

10/524 275

EAST Search History

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	16774	luciferase and transgenic	US-PGPUB; USPAT; DERWENT	ADJ	ON	2007/03/01 11:56
L2	9722	luciferase and (transgenic animal)	US-PGPUB; USPAT; DERWENT	ADJ	ON	2007/03/01 11:56
L3	53	luciferase.ab. and (transgenic animal)	US-PGPUB; USPAT; DERWENT	ADJ	ON	2007/03/01 11:56
L4	32	luciferase.ab. and (transgenic animal).ab.	US-PGPUB; USPAT; DERWENT	ADJ	ON	2007/03/01 12:11
L5	57	luciferase.ab. and (plant).ab.	US-PGPUB; USPAT; DERWENT	ADJ	ON	2007/03/01 12:11

500612 10/529275
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Set	Items	Description
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? s	luciferase and transgenic	
	19049	LUCIFERASE
	81855	TRANSGENIC
S1	986	LUCIFERASE AND TRANSGENIC
? s	luciferase and ((fusion or chimera?) and protein)	
	19049	LUCIFERASE
	106398	FUSION
	41289	CHIMER?
	1723605	PROTEIN
S2	913	LUCIFERASE AND ((FUSION OR CHIMER?) AND PROTEIN)
? s	s2 and plant	
	913	S2
	780150	PLANT
S3	25	S2 AND PLANT
? t	s3/7/1-25	

3/7/1

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18960896 BIOSIS NO.: 200600306291

The Arabidopsis Aux/IAA %protein% family has diversified in degradation
and auxin responsiveness

AUTHOR: Dreher Kate A; Brown Jessica; Saw Robert E; Callis Judy (Reprint)

AUTHOR ADDRESS: Univ Calif Davis, Plant Biol Grad Grp Program, Davis, CA
95616 USA**USA

AUTHOR E-MAIL ADDRESS: jcallis@ucdavis.edu

JOURNAL: Plant Cell 18 (3): p699-714 MAR 2006 2006

ISSN: 1040-4651

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Rapid, auxin-responsive degradation of multiple
auxin/indole-3-acetic acid (Aux/IAA) proteins is essential for
%plant% growth and development. Domain II residues were previously
shown to be required for the degradation of several Arabidopsis thaliana
Aux/IAA proteins. We examined the degradation of additional full-length
family members and the proteolytic importance of N-terminal residues
outside domain II using %luciferase% (LUC) fusions. Elimination of
domain I did not affect degradation. However, substituting an Arg for a
conserved Lys between domains I and II specifically impaired basal
degradation without compromising the auxin-mediated acceleration of
degradation. IAA8, IAA9, and IAA28 contain domain II and a conserved Lys,
but they were degraded more slowly than previously characterized family
members when expressed as LUC fusions, suggesting that sequences outside
domain II influence proteolysis. We analyzed the degradation of IAA31,
with a region somewhat similar to domain II but without the conserved
Lys, and of IAA20, which lacks domain II and the conserved Lys. Both
IAA20: LUC and epitope-tagged IAA20 were long-lived, and their longevity

was not influenced by auxin. Epitope-tagged IAA31 was long-lived, like IAA20, but by contrast, it showed accelerated degradation in response to auxin. The existence of long-lived and auxin-insensitive Aux/IAA proteins suggests that they may play a novel role in auxin signaling.

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18763751 BIOSIS NO.: 200600109146

PIF1 is regulated by light-mediated degradation through the ubiquitin-26S proteasome pathway to optimize photomorphogenesis of seedlings in Arabidopsis

AUTHOR: Shen Hui; Moon Jennifer; Huq Enamul (Reprint)

AUTHOR ADDRESS: Univ Texas, Sect Mol Cell and Dev Biol, Austin, TX 78712
USA**USA

AUTHOR E-MAIL ADDRESS: huq@mail.utexas.edu

JOURNAL: Plant Journal 44 (6): p1023-1035 DEC 2005 2005

ISSN: 0960-7412

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Light signals perceived by the phytochrome (phy) family of sensory photoreceptors control multiple aspects of %%plant%% development. Recently, PIF1, a phy-interacting basic helix-loop-helix (bHLH) transcription factor, has been shown to negatively regulate facets of the photomorphogenesis of seedlings. Moreover, the transcriptional activation activity of PIF1 is reduced in a phy-dependent manner. In this study we use the %%luciferase%% (LUC) activity of the LUC-PIF1 %%fusion%% %%protein%% as an indicator of the stability of PIF1 in various light conditions. We found that the activity of LUC-PIF1 in both transient and stable transgenic lines is rapidly reduced in light, while the LUC-only control is stable under the same conditions, suggesting that PIF1 is degraded in response to light. Fluence-rate response curves indicate that PIF1 degradation is very sensitive to the quality and quantity of light. The half-life of PIF1 is about 16 min under 10 μ mol m⁻² sec⁻¹ red light. PIF1 reaccumulates in the subsequent dark period after light-induced degradation, signifying that PIF1 not only functions in the dark and during the transition from etiolated to de-etiolated growth, but may also function during diurnal cycles. Inhibitors of the 26S proteasome increased the stability of PIF1, indicating that degradation of PIF1 is mediated by the ubiquitin-26S proteasome pathway. Further, de novo %%protein%% synthesis is not required for degradation of PIF1, as the presence of cycloheximide does not prevent degradation of PIF1 in the light. Taken together, these results suggest that the light signals perceived by phys induce the degradation of PIF1 and other phy-interacting factors to optimize photomorphogenesis.

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18681054 BIOSIS NO.: 200600026449

The zinc-finger %%protein%% Zat12 plays a central role in reactive oxygen

and abiotic stress signaling in Arabidopsis
AUTHOR: Davletova Sholpan; Schlauch Karen; Coutu Jesse; Mittler Ron
(Reprint)
AUTHOR ADDRESS: Univ Nevada, Dept Biochem and Mol Biol, Reno, NV 89557 USA
**USA
AUTHOR E-MAIL ADDRESS: ronm@unr.edu
JOURNAL: Plant Physiology (Rockville) 139 (2): p847-856 OCT 2005 2005
ISSN: 0032-0889
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: **Plant** acclimation to environmental stress is controlled by a complex network of regulatory genes that compose distinct stress-response regulons. In contrast to many signaling and regulatory genes that are stress specific, the zinc-finger **protein** Zat12 responds to a large number of biotic and abiotic stresses. Zat12 is thought to be involved in cold and oxidative stress signaling in Arabidopsis (*Arabidopsis thaliana*); however, its mode of action and regulation are largely unknown. Using a **fusion** between the Zat12 promoter and the reporter gene **luciferase**, we demonstrate that Zat12 expression is activated at the transcriptional level during different abiotic stresses and in response to a wound-induced systemic signal. Using Zat12 gain- and loss-of-function lines, we assign a function for Zat12 during oxidative, osmotic, salinity, high light, and heat stresses. Transcriptional profiling of Zat12-overexpressing plants and wild-type plants subjected to H₂O₂ stress revealed that constitutive expression of Zat12 in Arabidopsis results in the enhanced expression of oxidative- and light stress-response transcripts. Under specific growth conditions, Zat12 may therefore regulate a collection of transcripts involved in the response of Arabidopsis to high light and oxidative stress. Our results suggest that Zat12 plays a central role in reactive oxygen and abiotic stress signaling in Arabidopsis.

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18332735 BIOSIS NO.: 200510027235
Analysis of **chimeric** RGS proteins in yeast for the functional evaluation of **protein** domains and their potential use in drug target validation
AUTHOR: Ajit Seena K; Young Kathleen H (Reprint)
AUTHOR ADDRESS: Wyeth Ayerst Res, Neurosci Discovery Res, CN 8000, Princeton, NJ 08543 USA**USA
AUTHOR E-MAIL ADDRESS: youngk3@wyeth.com
JOURNAL: Cellular Signalling 17 (7): p817-825 JUL 05 2005
ISSN: 0898-6568
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: For the identification of regulators of G-**protein** signaling (RGS) modulators, previously, we developed a **luciferase** based yeast pheromone response (YPhR) assay to functionally investigate RGS4 (K.H. Young, Y. Wang, C. Bender, S. Ajit, F. Ramirez, A. Gilbert, B.W

Nieuwenhuijsen, in: D.P. Siderovski (Ed.), Meth. Enzymol. 389 Regulators of G-~~protein~~ Signaling, Part A, 2004.). To extend the diversity of this assay, additional RGS proteins were evaluated for functional complementation in a RGS (sst2 Delta) knockout yeast strain. For RGS proteins that did not function in their native form, a series of ~~chimeric~~ constructs were generated with the N terminus of RGS4 fused in frame with the partial or full-length RGS cDNA of interest. RGS4 N terminus fused to either full-length or the C terminus of RGS7 successfully complemented sst2 Delta. On the contrary, the RGS7N/RGS4C ~~chimera~~ (N terminus of RGS7 in frame with RGS domain of RGS4) was not effective, showing that N terminus of RGS4 helps in targeting. RGS 10 exists as two splice variants, differing only by 8 amino acids (aa) in the N terminus, being either 168 aa (RGS10S), or 174 aa (RGS10). While RGS10 was functional in yeast, RGS10S required the presence of the N terminus of RGS4 for its activity. Although the same RGS4 N terminus domain was present in ~~chimeras~~ generated, the GTPase accelerating ~~protein~~ (GAP) function observed was not similar, suggesting differences in the RGS domain function. In conclusion, the use of RGS4 N terminus ~~chimeric~~ constructs enabled us to develop a selectivity assay for different RGS proteins. (c) 2004 Elsevier Inc. All rights reserved.

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17615797 BIOSIS NO.: 200300584516

Anti-IgM-induced down-regulation of nuclear Thy28 ~~protein~~ expression in Ramos B lymphoma cells.

AUTHOR: Jiang X Z; Toyota H; Yoshimoto T; Takada E; Asakura H; Mizuguchi J (Reprint)

AUTHOR ADDRESS: Department of Immunology, Tokyo Medical University, 6-1-1 Shinjuku, Shinjuku-ku, Tokyo, 160-8402, Japan**Japan

AUTHOR E-MAIL ADDRESS: mizu@tokyo-med.ac.jp

JOURNAL: Apoptosis 8 (5): p509-519 October 2003 2003

MEDIUM: print

ISSN: 1360-8185 (ISSN print)

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: We recently cloned mouse Thy28 cDNA (mThy28), which is highly conserved among vertebrates and plants. The mThy28 mRNA is highly expressed in testis, liver, kidney, brain, with moderate expression in thymus, spleen, and heart. In the present study, characteristics of mouse Thy28 ~~protein~~ expression were examined using rabbit anti-mThy28 polyclonal antibody (Ab). Levels of mThy28 ~~protein~~ expression were highest in testis, with moderate expression in liver, spleen, and thymus. The Thy28 ~~protein~~ was mainly located in the nucleus, which was revealed by immunofluorescence microscopy and Western blotting using anti-mThy28 Ab, and transient expression of the mThy28/EGFP ~~fusion~~ gene. Engagement of membrane immunoglobulin with anti-IgM induced down-regulation of human Thy28 expression at both mRNA and ~~protein~~ levels, accompanied by induction of apoptosis in Ramos B lymphoma cells. Expression of ~~protein~~ and mRNA and induction of apoptosis were evaluated by flow cytometry/Western blotting, reverse

transcription-polymerase chain reaction, and propidium iodide staining, respectively. Anti-IgM also down-regulated the promoter activity of the mThy28 gene, as demonstrated by **luciferase** assay. Thus, the anti-IgM-induced down-regulation of the nuclear Thy28 expression appears to correlate with the induction of apoptosis in Ramos B lymphoma cells.

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17463091 BIOSIS NO.: 200300418753

Targeted activation tagging of the Arabidopsis NBS-LRR gene, ADR1, conveys resistance to virulent pathogens.

AUTHOR: Grant John J; Chini Andrea; Basu Debrabata; Loake Gary J (Reprint)

AUTHOR ADDRESS: Institute of Cell and Molecular Biology, University of Edinburgh, King's Buildings, Edinburgh, EH9 3JR, UK**UK

AUTHOR E-MAIL ADDRESS: gloake@srv0.bio.ed.ac.uk

JOURNAL: Molecular Plant-Microbe Interactions 16 (8): p669-680 August 2003
2003

MEDIUM: print

ISSN: 0894-0282 (ISSN print)

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: A transgenic Arabidopsis line containing a **chimeric** PR-1:: **luciferase** (LUC) reporter gene was subjected to mutagenesis with activation tags. Screening of lines via high-throughput LUC imaging identified a number of dominant Arabidopsis mutants that exhibited enhanced PR-1 gene expression. Here, we report the characterization of one of these mutants, designated activated disease resistance (adr) 1. This line showed constitutive expression of a number of key defense marker genes and accumulated salicylic acid but not ethylene or jasmonic acid. Furthermore, adr1 plants exhibited resistance against the biotrophic pathogens *Peronospora parasitica* and *Erysiphe cichoracearum* but not the necrotrophic fungus *Botrytis cinerea*. Analysis of a series of adr1 double mutants suggested that adr1-mediated resistance against *P. parasitica* was salicylic acid (SA)-dependent, while resistance against *E. cichoracearum* was both SA-dependent and partially NPR1-dependent. The ADR1 gene encoded a **protein** possessing a number of key features, including homology to subdomains of **protein** kinases, a nucleotide binding domain, and leucine-rich repeats. The controlled, transient expression of ADR1 conveyed striking disease resistance in the absence of yield penalty, highlighting the potential utility of this gene in crop protection.

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17421422 BIOSIS NO.: 200300378699

Gene trapping of the Arabidopsis genome with a firefly **luciferase** reporter.

AUTHOR: Yamamoto Yoshiharu Y; Tsuchihara Yumi; Gohda Kazuhito; Suzuki Kumiko; Matsui Minami (Reprint)

AUTHOR ADDRESS: Plant Function Exploration Team, Plant Functional Genomics
Research Group, Genomic Sciences Center, RIKEN Yokohama Institute, 1-7-22
Suyehiro-cho, Tsurumi-ku, Yokohama, Kanagawa, 230-0045, Japan**Japan
AUTHOR E-MAIL ADDRESS: minami@postman.riken.go.jp
JOURNAL: Plant Journal 35 (2): p273-283 July 2003 2003
MEDIUM: print
ISSN: 0960-7412
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Experiments with gene-trap vectors containing the firefly
%%luciferase%% (LUC) reporter genes were carried out with the aim of
analyzing functions of the Arabidopsis genome. Studies with %%protein%%
%%fusion%%-type trap vectors as well as an internal ribosome entry site
(IRES)-assisted non-%%fusion%%-type vector revealed that both types of
vectors were suitable for gene trapping in Arabidopsis, although there
were some differences in trapping efficiencies. The established trap
lines were subjected to analyses for light responses, demonstrating the
powerful and unique applications of a LUC-trapping system. A systematic
survey of the insertion sites of the T-DNAs in LUC-expressing lines
revealed 12-41% gene-trapping efficiencies depending on the vector. We
demonstrate that the LUC-trapping system provides a unique system with
which to monitor temporal expression of %%plant%% genes.

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17205873 BIOSIS NO.: 200300164592
The Arabidopsis luel mutant defines a katanin p60 ortholog involved in
hormonal control of microtubule orientation during cell growth.
AUTHOR: Bouquin Thomas; Mattsson Ole; Naested Henrik; Foster Randy; Mundy
John (Reprint)
AUTHOR ADDRESS: Department of Plant Physiology, Institute of Molecular
Biology, Oster Farimagsgade 2A, 1353K, Copenhagen, Denmark**Denmark
AUTHOR E-MAIL ADDRESS: mundy@biobase.dk
JOURNAL: Journal of Cell Science 116 (5): p791-801 March 1, 2003 2003
MEDIUM: print
ISSN: 0021-9533 _(ISSN print)
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The luel mutant was previously isolated in a bio-imaging screen
for Arabidopsis mutants exhibiting inappropriate regulation of an
AtGA20ox1 promoter-%%luciferase%% reporter %%fusion%%. Here we show
that luel is allelic to fra2, bot1 and erh3, and encodes a truncated
katanin-like microtubule-severing %%protein%% (AtKSS). Complementation
of luel with the wild-type AtKSS gene restored both wild-type stature and
%%luciferase%% reporter levels. Hormonal responses of luel to ethylene
and gibberellins revealed inappropriate cortical microtubule
reorientation during cell growth. Moreover, a %%fusion%% between the
AtKSS %%protein%% and GFP decorated cortical microtubules. A yeast
two-hybrid screen with AtKSS as the bait identified proteins related to
those involved in microtubule processing, including a katanin p80 subunit

and a kinesin ortholog. These results indicate that AtKSS is involved in microtubule dynamics in response to %%%plant%%% hormones.

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17164503 BIOSIS NO.: 200300121613

Regulation of gene expression in Arabidopsis thaliana by artificial zinc finger %%%chimeras%%%.

AUTHOR: Sanchez Juan-Pablo (Reprint); Ullman Chris; Moore Michael; Choo Yen ; Chua Nam-Hai

AUTHOR ADDRESS: Laboratory of Plant Molecular Biology, Rockefeller University, 1230 York Avenue, New York, NY, 10021, USA**USA

AUTHOR E-MAIL ADDRESS: chua@rockvax.rockefeller.edu

JOURNAL: Plant and Cell Physiology 43 (12): p1465-1472 December 2002 2002

MEDIUM: print

ISSN: 0032-0781 (ISSN print)

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: The artificial regulation of endogenous gene expression in plants is limited to only a few approaches. Here, we describe the use of artificial zinc finger %%%chimeras%%% to regulate the expression of a known reporter construct. The artificial zinc finger %%%chimera%%% TFIIIAZif is a %%%fusion%%% %%%protein%%% consisting of the four zinc fingers of TFIIIA linked through a spacer region to the three zinc fingers of Zif268. This artificial zinc finger %%%chimera%%% is able to bind specifically to a target DNA sequence (ZBS, zinc finger binding site) of 27 base pairs (bp). TFIIIAZif was fused to a transactivation domain from the herpes simplex virus VP16 or its tetramer VP64 to give ZF-VP16 or ZF-VP64, respectively. In transient expression assays, these two transcription activators were able to activate a target reporter gene (luc and GFP) expressed from a minimal -46 35S promoter linked to four copies of ZBS. The activation was confirmed in transgenic plants using an inducible XVE system (Zuo et al. (2000) %%%Plant%%% J. 24: 265) to express ZF-VP16 or ZF-VP64. Furthermore, to test the specificity of ZF-VP64 we have compared reporter gene expression from a wild type (1XZBS) and a mutant (1XZBSmu) binding site in transgenic plants. The 1XZBS was used to express green fluorescent %%%protein%%% (GFP) whereas the 1XZBSmu was used to express red fluorescent %%%protein%%% (RFP). Upon induction of ZF-VP64 we found a much higher expression of GFP (about 33-fold) as compared to RFP expression. These results suggest that artificial zinc finger %%%chimeras%%% can be used to target specific DNA sequences and to regulate gene expression in plants.

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16946764 BIOSIS NO.: 200200540275

The circadian clock that controls gene expression in Arabidopsis is tissue specific

AUTHOR: Thain Simon C; Murtas Giovanni; Lynn James R; McGrath Robert B;

Millar Andrew J (Reprint)
AUTHOR ADDRESS: Department of Biological Sciences, University of Warwick,
Coventry, CV4 7AL, UK**UK
JOURNAL: Plant Physiology (Rockville) 130 (1): p102-110 September, 2002
2002
MEDIUM: print
ISSN: 0032-0889
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The expression of CHALCONE SYNTHASE (CHS) expression is an important control step in the biosynthesis of flavonoids, which are major photoprotectants in plants. CHS transcription is regulated by endogenous programs and in response to environmental signals. %%Luciferase%% reporter gene fusions showed that the CHS promoter is controlled by the circadian clock both in roots and in aerial organs of transgenic Arabidopsis plants. The period of rhythmic CHS expression differs from the previously described rhythm of chlorophyll a/b-binding %%protein%% (CAB) gene expression, indicating that CHS is controlled by a distinct circadian clock. The difference in period is maintained in the wild-type Arabidopsis accessions tested and in the de-etiolated 1 and timing of CAB expression 1 mutants. These clock-affecting mutations alter the rhythms of both CAB and CHS markers, indicating that a similar (if not identical) circadian clock mechanism controls these rhythms. The distinct tissue distribution of CAB and CHS expression suggests that the properties of the circadian clock differ among %%plant%% tissues. Several animal organs also exhibit heterogeneous circadian properties in culture but are believed to be synchronized in vivo. The fact that differing periods are manifest in intact plants supports our proposal that spatially separated copies of the %%plant%% circadian clock are at most weakly coupled, if not functionally independent. This autonomy has apparently permitted tissue-specific specialization of circadian timing.

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16842282 BIOSIS NO.: 200200435793
%%Plant%%-specific promoter sequences carry elements that are recognised by the eubacterial transcription machinery
AUTHOR: Jacob Daniela; Lewin Astrid (Reprint); Meister Beate; Appel Bernd
AUTHOR ADDRESS: Robert Koch-Institut, Nordufer 20, 13353, Berlin, Germany**
Germany
JOURNAL: Transgenic Research 11 (3): p291-303 June, 2002 2002
MEDIUM: print
ISSN: 0962-8819
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: During evolution the promoter elements from prokaryotes and eukaryotes have developed differently with regard to their sequence and structure, implying that in general a transfer of eukaryotic promoter sequences into prokaryotes will not cause an efficient gene expression. However, there have been reports on the functionality of the 35S promoter

from cauliflower mosaic virus (CaMV) in bacteria. We therefore decided to experimentally investigate the capability of **%%plant%%** promoter sequences to direct gene expression in various bacteria. Accordingly, we tested ten different **%%plant%%**-specific promoters from *Solanum tuberosum*, *Nicotiana tabacum*, CaMV, *Agrobacterium tumefaciens*, and *A. rhizogenes* for their ability to initiate transcription in five different eubacterial species (*Escherichia coli*, *Yersinia enterocolitica*, *A. tumefaciens*, *Pseudomonas putida*, and *Acinetobacter* sp. BD413). To monitor the strength of the **%%plant%%**-specific promoters in bacteria we created fusions between these promoters and the coding region of the **%%luciferase%%** genes from *Vibrio harveyi* and measured the luminescence in the bacteria. Heterologous gene expression was observed in 50% of the combinations analysed. We then mapped the transcription start site caused by one of the **%%plant%%**-specific promoters, the ST-LS1 promoter from *S. tuberosum*, in these bacterial species. The location of the mapped transcription start site indicated that the sequences of the **%%plant%%** promoter themselves were recognised by the bacterial transcription apparatus. The recognition of **%%plant%%**-specific promoter sequences by the bacterial RNA polymerase was further confirmed by site-directed mutagenesis of the ST-LS1 promoter and the analysis of the effects of the mutations on the strength of gene expression in *E. coli*. Using these mutants in our reporter assays we could localise the sequences of the ST-LS1 promoter serving as -10 region in *E. coli*. The results of our study show that promoter sequences are much less specific than is generally assumed. This is of great importance for our knowledge about the evolution of gene expression systems and for the construction of optimised expression vectors.

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16813706 BIOSIS NO.: 200200407217

Jasmonate response locus JAR1 and several related Arabidopsis genes encode enzymes of the firefly **%%luciferase%%** superfamily that show activity on jasmonic, salicylic, and indole-3-acetic acids in an assay for adenylation

AUTHOR: Staswick Paul E (Reprint); Tiryaki Iskender; Rowe Martha L

AUTHOR ADDRESS: Department of Agronomy and Horticulture, University of Nebraska, Lincoln, NE, 68583, USA**USA

JOURNAL: Plant Cell 14 (6): p1405-1415 June, 2002 2002

MEDIUM: print

ISSN: 1040-4651

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Jasmonic acid (JA) and related cyclopentanones are critical **%%plant%%** signaling molecules, but their mode of action at the molecular level is unclear. A map-based approach was used to identify the defective gene in the Arabidopsis JA response mutant jar1-1. JAR1 is 1 of 19 closely related Arabidopsis genes that are similar to the auxin-induced soybean GH3 gene. Analysis of fold predictions for this **%%protein%%** family suggested that JAR1 might belong to the acyl adenylate-forming firefly **%%luciferase%%** superfamily. These enzymes activate the carboxyl groups of a variety of substrates for their

subsequent biochemical modification. An ATP-PPi isotope exchange assay was used to demonstrate adenylation activity in a glutathione S-transferase-JAR1.***fusion*** ***protein***. Activity was specific for JA, suggesting that covalent modification of JA is important for its function. Six other Arabidopsis genes were specifically active on indole-3-acetic acid (IAA), and one was active on both IAA and salicylic acid. These findings suggest that the JAR1 gene family is involved in multiple important ***plant*** signaling pathways.

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16765001 BIOSIS NO.: 200200358512

The 5'-untranslated region of the ntp303 gene strongly enhances translation during pollen tube growth, but not during pollen maturation

AUTHOR: Hultink Raymond J M; de Groot Peter F M; Croes Anton F; Quaedvlieg William; Twell Dave; Wullems George J; van Herpen Marinus M A (Reprint)

AUTHOR ADDRESS: Department of Experimental Botany, Plant Genetics, Catholic University Nijmegen, Toernooiveld 1, 6525 ED, Nijmegen, Netherlands** Netherlands

JOURNAL: Plant Physiology (Rockville) 129 (1): p342-353 May, 2002 2002

MEDIUM: print

ISSN: 0032-0889

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Transcripts of the ntp303 gene accumulate abundantly throughout pollen development, whereas the ***protein*** only accumulates to detectable levels after pollen germination. In an attempt to explain the divergence in the accumulation profiles of the mRNA and the ***protein***, we investigated the role of the untranslated regions (UTRs) in enhancing ntp303 translation during the transition from developing to germinating pollen. ***Luciferase*** reporter gene ***fusion*** constructs containing the ntp303 5'-UTR gave rise to ***luciferase*** activity that was up to 60-fold higher during pollen tube growth than that of constructs containing different 5'-UTRs. No apparent differences in the ***luciferase*** activity of these constructs were observed during pollen development. The ntp303 5'-UTR-mediated increase in ***luciferase*** activity was not significantly influenced by coding region or 3'-UTR sequences. Furthermore, enhanced ***luciferase*** activity directed by the ntp303 5'-UTR occurred predominantly at the post-transcriptional level. A series of 5'-UTR deletion constructs was created to identify putative regulatory sequences required for the high level of translation during pollen tube growth. Two predicted stem loop structures (H-I and H-II) caused a complete inhibition of the enhanced translation after their total or partial deletion. A (GAA)8 repeat within the H-I stem loop structure was demonstrated to be important for the modulation of translation efficiency. The H-II stem loop structure was found to be essential for the determination of mRNA stability.

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16664179 BIOSIS NO.: 200200257690

Activation of the promoters of Arabidopsis genes for the branched-chain
alpha-keto acid dehydrogenase complex in transgenic tobacco BY-2 cells
under sugar starvation

AUTHOR: Fujiki Yuki (Reprint); Ito Masaki; Itoh Takashi; Nishida Ikuo;
Watanabe Akira

AUTHOR ADDRESS: Pflanzenphysiologie, ZMBP, Universitaet Tuebingen, Auf der
Morgenstelle 1, D-72076, Tuebingen, Germany**Germany

JOURNAL: Plant and Cell Physiology 43 (3): p275-280 March, 2002 2002

MEDIUM: print

ISSN: 0032-0781

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Sugar starvation exerted by sub-10 mM levels of sucrose on
Arabidopsis T87 suspension-cultured cells triggered marked accumulation
of the transcripts of genes for Elbeta and E2 subunit of the
branched-chain alpha-keto acid dehydrogenase complex. Similar levels of
sugar starvation increased the **luciferase** activity in transgenic
tobacco BY-2 lines expressing the Arabidopsis Elbeta- or E2-promoter-
luciferase **fusion** gene. These results indicate that sugar
levels tightly regulate the Elbeta and E2 promoter activity in the
heterologous **plant** system. We further showed in the transgenic
tobacco BY-2 lines that sugar-starvation-induced activation of the Elbeta
and E2 promoters was prevented by K-252a, an inhibitor of Ser/Thr
protein kinase, and was enhanced by okadaic acid, an inhibitor of
protein phosphatases. By contrast, the cauliflower mosaic virus 35S
promoter activity in sugar-starved BY-2 cells was not significantly
affected by K-252a and only slightly enhanced by okadaic acid. Taken
together, we propose that transcriptional activation of genes for the
branched-chain alpha-keto acid dehydrogenase complex and its modulation
by specific **protein** kinases/phosphatases are of critical importance
in branched-chain amino acid catabolism in **plant** cells under sugar
starvation.

3/7/15

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16628024 BIOSIS NO.: 200200221535

Fusion genetic analysis of jasmonate-signalling mutants in
Arabidopsis

AUTHOR: Jensen Anders B; Raventos Dora; Mundy John (Reprint)

AUTHOR ADDRESS: Institute of Molecular Biology, University of Copenhagen,
Oster Farimagsgade 2A, DK-1353, Copenhagen K, Denmark**Denmark

JOURNAL: Plant Journal 29 (5): p595-606 March, 2002 2002

MEDIUM: print

ISSN: 0960-7412

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Jasmonates induce **plant**-defence responses and act to
regulate defence-related genes including positive feedback of the

lipoxygenase 2 (LOX2) gene involved in jasmonate synthesis. To identify jasmonate-signalling mutants, we used a **fusion** genetic strategy in which the firefly **luciferase** (FLUC) and Escherichia coli beta-glucuronidase (GUS) reporters were expressed under control of the jasmonate-responsive LOX2 promoter. Spatial and temporal patterns of reporter expression were determined initially, and revealed that JA-responsive expression from the LOX2 promoter required de novo **protein** synthesis. Reporter activity was also induced by the **protein** kinase inhibitor staurosporine and antagonized by the **protein** phosphatase inhibitor okadaic acid. FLUC bio-imaging, RNA gel-blot analysis and progeny analyses identified three recessive mutants that underexpress that FLUC reporter, designated juel, 2 and 3, as well as two recessive mutants, designated joel and 2, that overexpress the reporter. Genetic analysis indicated that reporter overexpression in the joe mutants requires COI, joel responded to MeJA with increased anthocyanin accumulation, while joe2 responded with decreased root growth inhibition. In addition, reporter induction and endogenous LOX2 expression by staurosporine was absent in joe2.

3/7/16

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16536776 BIOSIS NO.: 200200130287

AUX/IAA proteins are active repressors, and their stability and activity are modulated by auxin

AUTHOR: Tiwari Shiv B; Wang Xiao-Jun; Hagen Gretchen; Guilfoyle Tom J
(Reprint)

AUTHOR ADDRESS: Department of Biochemistry, University of Missouri, 117
Schweitzer Hall, Columbia, MO, 65211, USA**USA

JOURNAL: Plant Cell 13 (12): p2809-2822 December, 2001 2001

MEDIUM: print

ISSN: 1040-4651

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Aux/IAA genes are early auxin response genes that encode short-lived nuclear proteins with four conserved domains, referred to as I, II, III, and IV. Arabidopsis Aux/IAA proteins repressed transcription on auxin-responsive reporter genes in protoplast transfection assays. Mutations in domain II resulted in increased repression, whereas mutations in domains I and III partially relieved repression. Aux/IAA proteins fused to a heterologous DNA binding domain were targeted to promoters of constitutively expressed reporter genes and actively repressed transcription in an auxin-responsive and dose-dependent manner. In comparison with an unfused **luciferase** **protein**, **luciferase** fused to Aux/IAA proteins displayed less **luciferase** activity, which further decreased in the presence of auxin in transfected protoplasts. Domain II mutations increased and domain I mutations decreased **luciferase** activity with the **fusion** proteins. These results suggested that Aux/IAA proteins function as active repressors by dimerizing with auxin response factors bound to auxin response elements and that early auxin response genes are regulated by auxin-modulated stabilities of Aux/IAA proteins.

3/7/17

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16368282 BIOSIS NO.: 200100540121

Rapid degradation of auxin/indoleacetic acid proteins requires conserved amino acids of domain II and is proteasome dependent

AUTHOR: Ramos Jason A; Zenser Nathan; Leyser Ottoline; Callis Judy (Reprint)

AUTHOR ADDRESS: Biochemistry and Molecular Biology Graduate Group, Section of Molecular and Cellular Biology, University of California, 1 Shields Avenue, Davis, CA, 95616, USA**USA

JOURNAL: Plant Cell 13 (10): p2349-2360 October, 2001 2001

MEDIUM: print

ISSN: 1040-4651

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Auxin rapidly induces auxin/indoleacetic acid (Aux/IAA) transcription. The proteins encoded are short-lived nucleus-localized transcriptional regulators that share four conserved domains. In a transient assay measuring protein accumulation, an Aux/IAA 13-amino acid domain II consensus sequence was sufficient to target firefly luciferase (LUC) for low protein accumulation equivalent to that observed previously for full-length PSIAA6. Single amino acid substitutions in these 13 amino acids, corresponding to known auxin response mutants, resulted in a sixfold to 20-fold increase in protein accumulation. Naturally occurring variant amino acids had no effect. Residues identified as essential by single alanine substitutions were not sufficient when all flanking amino acids were alanine, indicating the importance of flanking regions. Using direct protein degradation measurements in transgenic Arabidopsis seedlings, full-length IAA1, PSIAA6, and the N-terminal 73 PSIAA6 amino acids targeted LUC for rapid degradation with 8-min half-lives. The C-terminal 109 amino acids did not affect LUC half-life. Smaller regions containing domain II also targeted LUC for rapid degradation, but the rates were not equivalent to those of the full-length protein. A single domain II substitution in the context of full-length PSIAA6 increased half-life 30-fold. Proteasome inhibitors affected Aux/IAA::LUC fusion protein accumulation, demonstrating the involvement of the proteasome.

3/7/18

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15528095 BIOSIS NO.: 200000246408

Degradation of Aux/IAA proteins is essential for normal auxin signalling

AUTHOR: Worley Cathy K; Zenser Nathan; Ramos Jason; Rouse Dean; Leyser Ottoline; Theologis Athanasios; Callis Judy (Reprint)

AUTHOR ADDRESS: Department of Plant and Soil Science, University of Delaware, 149 Townsend Hall, Newark, DE, 19717, USA**USA

JOURNAL: Plant Journal 21 (6): p553-562 March, 2000 2000

MEDIUM: print

ISSN: 0960-7412
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The growth substance auxin mediates many cellular processes, including division, elongation and differentiation. PSIAA6 is a member of the Aux/IAA family of short-lived putative transcriptional regulators that share four conserved domains and whose mRNAs are rapidly induced in the presence of auxin. Here PSIAA6 was shown to serve as a dominant transferable degradation signal when present as a translational **fusion** with firefly **luciferase** (LUC), with an in vivo half-life of 13.5 min in transgenic Arabidopsis seedlings. In a transient assay system in tobacco protoplasts using steady-state differences as an indirect measure of **protein** half-life, LUC fusions with full-length PSIAA6 and IAA1, an Aux/IAA **protein** from Arabidopsis, resulted in **protein** accumulations that were 3.5 and 1.0%, respectively, of that with LUC alone. An N-terminal region spanning conserved domain II of PSIAA6 containing amino acids 18-73 was shown to contain the necessary cis-acting element to confer low **protein** accumulation onto LUC, while a **fusion** **protein** with PSIAA6 amino acids 71-179 had only a slight effect. Single amino acid substitutions of PSIAA6 in conserved domain II, equivalent to those found in two alleles of axr3, a gene that encodes Aux/IAA **protein** IAA17, resulted in a greater than 50-fold increase in **protein** accumulation. Thus, the same mutations resulting in an altered auxin response phenotype increase Aux/IAA **protein** accumulation, providing a direct link between these two processes. In support of this model, transgenic plants engineered to over-express IAA17 have an axr3-like phenotype. Together, these data suggest that rapid degradation of Aux/IAA proteins is necessary for a normal auxin response.

3/7/19
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15461178 BIOSIS NO.: 200000179491
Differential roles of the 5' untranslated regions of cucumber mosaic virus RNAs 1, 2, 3 and 4 in translational competition
AUTHOR: Kwon Chang Seob; Chung Won-Il (Reprint)
AUTHOR ADDRESS: Department of Biological Sciences, Korea Advanced Institute of Science and Technology, 373-1 Kusong-dong, Yusong-ku, Taejeon, 305-701, South Korea**South Korea
JOURNAL: Virus Research 66 (2): p175-185 Feb., 2000 2000
MEDIUM: print
ISSN: 0168-1702
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: RNA species of **plant** tripartite RNA viruses show distinct translational activities in vitro when the viral RNA concentration is high. However, it is not known what causes the differential translation of virion RNAs. Using an in vitro wheat germ translation system, we investigated the translation efficiencies and competitive activities of **chimeric** cucumber mosaic virus (CMV) RNAs that contained viral

untranslated regions (UTRs) and a **luciferase**-coding sequence. The **chimeric** RNAs exhibited distinct translation efficiencies and competitive activities. For example, the translation of **chimeric** CMV RNA 4 was about 40-fold higher than that of **chimeric** CMV RNA 3 in a competitive environment. The distinct translation resulted mainly from differences in competitive activities rather than translation efficiencies of the **chimeric** RNAs. The differential competitive activities were specified by viral 5' UTRs, but not by 3' UTRs or viral proteins. The competitive translational activities of the 5' UTRs were as follows: RNA 4 (coat **protein**) > RNAs 2 and 1 (2a and 1a **protein**, or replicase) > RNA 3 (3a **protein**).

3/7/20

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15331553 BIOSIS NO.: 200000049866

The circadian clock controls the expression pattern of the circadian input photoreceptor, phytochrome B

AUTHOR: Bogнар Laszlo Kozma; Hall Anthony; Adam Eva; Thain Simon C; Nagy Ferenc (Reprint); Millar Andrew J (Reprint)

AUTHOR ADDRESS: Biological Research Center of the Hungarian Academy of Sciences, Plant Biology Institute, H-6701, Szeged, Hungary**Hungary

JOURNAL: Proceedings of the National Academy of Sciences of the United States of America 96 (25): p14652-14657 Dec. 7, 1999 1999

MEDIUM: print

ISSN: 0027-8424

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Developmental and physiological responses are regulated by light throughout the entire life cycle of higher plants. To sense changes in the light environment, plants have developed various photoreceptors, including the red/far-red light-absorbing phytochromes and blue light-absorbing cryptochromes. A wide variety of physiological responses, including most light responses, also are modulated by circadian rhythms that are generated by an endogenous oscillator, the circadian clock. To provide information on local time, circadian clocks are synchronized and entrained by environmental time cues, of which light is among the most important. Light-driven entrainment of the Arabidopsis circadian clock has been shown to be mediated by phytochrome A (phyA), phytochrome B (phyB), and cryptochromes 1 and 2, thus affirming the roles of these photoreceptors as input regulators to the **plant** circadian clock. Here we show that the expression of PHYB::LUC reporter genes containing the promoter and 5' untranslated region of the tobacco NtPHYB1 or Arabidopsis AtPHYB genes fused to the **luciferase** (LUC) gene exhibit robust circadian oscillations in transgenic plants. We demonstrate that the abundance of PHYB RNA retains this circadian regulation and use a PHYB::Luc **fusion** **protein** to show that the rate of PHYB synthesis is also rhythmic. The abundance of bulk PHYB **protein**, however, exhibits only weak circadian rhythmicity, if any. These data suggest that photoreceptor gene expression patterns may be significant in the daily regulation of **plant** physiology and indicate an unexpectedly intimate relationship between the components of the input pathway and the putative circadian clock mechanism in higher plants.

3/7/21

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15156330 BIOSIS NO.: 199900415990

Isolation and expression of an elongation-dependent gene of mung bean

(*Vigna radiata*) hypocotyl

AUTHOR: Hashimoto Hiroshi; Yamamoto Kotaro T (Reprint)

AUTHOR ADDRESS: Division of Biological Science, Graduate School of
Environmental Earth Science, Hokkaido University, Sapporo, 060-0810,
Japan**Japan

JOURNAL: Physiologia Plantarum 106 (2): p224-231 June, 1999 1999

MEDIUM: print

ISSN: 0031-9317

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: A cDNA clone of an auxin up-regulated gene, ARG8, was isolated from hypocotyl sections of etiolated mung bean (*Vigna radiata* (L.) Wilczek) seedlings by differential screening. The deduced amino acid sequence suggested that ARG8 may encode a cell wall %%%protein%%%. The steady state mRNA level of ARG8 increased by treatment of hypocotyl sections not only with indole-3-acetic acid (IAA) but also with fusaric acid, and the auxin inducibility was inhibited by the addition of 0.3 M mannitol in the incubation medium. This indicated that it was not auxin but elongation that regulated the expression of ARG8. The promoter activity of the 5'-flanking region of ARG8 was determined by assaying the transient expression of a %%%luciferase%%% %%%fusion%%% gene that was introduced into mung bean hypocotyl sections by the particle bombardment technique. The basal activity of the ARG8 upstream region was about a few tenths of that of a modified cauliflower mosaic virus 35S promoter, and it was increased a few fold by treatment with IAA. The auxin inducibility was completely suppressed by the addition of mannitol. A 5'-deletion analysis showed that a 53-bp region in the ARG8 promoter was important for the basal and elongation-dependent promoter activities.

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14283675 BIOSIS NO.: 199800077922

Expression of cholera toxin B subunit oligomers in transgenic potato plants

AUTHOR: Arakawa Takeshi; Chong Daniel K X; Merritt J Lawrence; Langridge
William H R (Reprint)

AUTHOR ADDRESS: Cent. Mol. Biol. Gene Therapy, Loma Linda Univ., Loma
Linda, CA 92350, USA**USA

JOURNAL: Transgenic Research 6 (6): p403-413 Nov., 1997 1997

MEDIUM: print

ISSN: 0962-8819

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: A gene encoding the cholera toxin B subunit **protein** (CTB), fused to an endoplasmic reticulum (ER) retention signal (SEKDEL) was inserted adjacent to the bidirectional mannopine synthase P2 promoter in a **plant** expression vector containing a bacterial **luciferase** AB **fusion** gene (luxF) linked to the P1 promoter. Potato leaf explants were transformed by *Agrobacterium tumefaciens* carrying the vector and kanamycin-resistant plants were regenerated. The CTB-SEKDEL **fusion** gene was identified in the genomic DNA of bioluminescent plants by polymerase chain reaction amplification. Immunoblot analysis indicated that **plant**-derived CTB **protein** was antigenically indistinguishable from bacterial CTB **protein**, and that oligomeric CTB molecules (Mr approx 50 kDa) were the dominant molecular species isolated from transgenic potato leaf and tuber tissues. Similar to bacterial CTB, **plant** synthesized CTB dissociated into monomers (Mr approx 15 kDa) during heat or acid treatment. The maximum amount of CTB **protein** detected in auxin-induced transgenic potato leaf and tuber tissues was approximately 0.3% of total soluble **plant** **protein**. Enzyme-linked immunosorbent assay methods indicated that **plant** -synthesized CTB **protein** bound specifically to GM1-ganglioside, the natural membrane receptor of cholera toxin. In the presence of the SEKDEL signal, CTB **protein** accumulates in potato tissues and is assembled into an oligomeric form that retains native biochemical and immunological properties. The expression of oligomeric CTB **protein** with immunological and biochemical properties identical to native CTB **protein** in edible plants opens the way for preparation of inexpensive food **plant**-based oral vaccines for protection against cholera and other pathogens in endemic areas throughout the world.

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13181037 BIOSIS NO.: 199698648870

Multiple DNA-**protein** complexes at a circadian-regulated promoter element

AUTHOR: Carre Isabelle A; Kay Steve A (Reprint)

AUTHOR ADDRESS: Natl. Sci. Found. Cent. Biol. Timing, Univ. Va., Charlottesville, VA 22903, USA**USA

JOURNAL: Plant Cell 7 (12): p2039-2051 1995 1995

ISSN: 1040-4651

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Higher **plant** CAB genes encode chlorophyll a/b binding proteins that are part of light-harvesting complexes in chloroplasts. Transcription of the Arabidopsis CAB2 (lhcb1*1) gene is under the control of a circadian oscillator and exhibits high amplitude diurnal oscillations that persist within a period close to 24 hr in the absence of environmental time cues. Initial deletion studies in transgenic tobacco have demonstrated that the region between -111 and -38 of the CAB2 promoter sequence confers circadian regulation to a **luciferase** (luc) reporter gene. We dissected this element further and characterized five DNA binding complexes from Arabidopsis whole-cell extracts that bind within this region of the promoter and may be components of the signal transduction pathway for the control of transcription by the circadian

clock. The in vivo analysis of cab2::luc %%%fusion%% constructs in transgenic Arabidopsis demonstrated that a circadian-regulated element lies within a 36-bp sequence that overlaps a conserved CCAAT box and contains binding sites for three putative transcription factors.

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11748031 BIOSIS NO.: 199395050297

Enhanced stable expression of a Vibrio %%%luciferase%% under the control of the OMEGA translational enhancer in transgenic plants

AUTHOR: Okumura K; Chlumsky L; Baldwin T O; Kado C I (Reprint)

AUTHOR ADDRESS: Dep. Plant Pathol., Univ. Calif., Davis, Calif. 95616, USA

**USA

JOURNAL: World Journal of Microbiology and Biotechnology 8 (6): p638-644 1992

ISSN: 0959-3993

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: A %%%fusion%% gene using luxA and luxB genes of Vibrio species (Vibrio fischeri and Vibrio harveyi) has been designed to express light autonomously in plants. LuxA:luxB was introduced into (tobacco) plants by a high-efficiency transformation system consisting of a high-copy virulence helper plasmid pUCD2614 and T-vector pUCD2715 containing luxA:luxB. The expression of luxA:luxB %%%fusion%% gene was optimized by adjusting the spacing between the genes and by placing the translational efficiency of its mRNA under the control of the OMEGA-3 translational enhancer. The resulting transgenic plants synthesized %%%luciferase%% at levels greater than 1% of the total leaf %%%protein%%. These plants produced light autonomously and light intensity was enhanced by the addition of aldehyde. That the luxA:luxB %%%fusion%% has been optimized enables its use as a reporter for gene activity in plants during development and under various stress-inducing conditions. These results show that a specific %%%protein%% from an introduced foreign gene can be produced with high efficiency in cultivated plants and such a system is therefore amenable for production of desired proteins through conventional farming methods.

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09770068 BIOSIS NO.: 198988085183

UTILIZATION OF %%%LUCIFERASE%% %%%FUSION%% GENES TO MONITOR DIFFERENTIAL REGULATION OF PHYTOHEMAGGLUTININ AND PHASEOLIN PROMOTERS IN TRANSGENIC TOBACCO

AUTHOR: RIGGS C D (Reprint); HUNT D C; LIN J; CHRISPEELS M J

AUTHOR ADDRESS: DEP BIOL, CENT MOL GENETICS, UNV CALIF, SAN DIEGO LA JOLLA, CALIF 92093-1116, USA**USA

JOURNAL: Plant Science (Shannon) 63 (1): p47-58 1989

ISSN: 0168-9452

DOCUMENT TYPE: Article

RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: The firefly **luciferase** gene can be used as a very sensitive and easily assayed reporter to study promoter activity in transgenic plants. To study the regulation of the promoters of the genes for phytohemagglutinin-L and β -phaseolin, two seed storage proteins of the common bean, *Phaseolus vulgaris*, we constructed **chimeric** genes consisting of their 5' flanking regions and the **luciferase** gene, and introduced the constructs into tobacco via *Agrobacterium*-mediated transformation. Our studies indicate that the two seed **protein** promoters are activated during seed development, and **luciferase** accumulates until seed maturation. Little or no **luciferase** activity could be found in other **plant** organs, and activity rapidly declined during seedling growth. The construct driven by the phaseolin promoter gave rise to about eight times more **luciferase** than the one driven by the phytohemagglutinin promoter. Thus, in addition to proper temporal and spatial regulation of promoter activity, we found that the differences in the levels of **luciferase** driven by the two promoters were similar to the calculated differences in the levels of **protein** in the bean resulting from the activities of these promoters. However, both promoters gave rise to similar levels of **luciferase** mRNA in the seeds of the transgenic plants. These results show that the levels of **luciferase** activity reflect not only the strength of the promoter but also the stability and/or translatability of the mRNA.

? ds

Set	Items	Description
S1	986	LUCIFERASE AND TRANSGENIC
S2	913	LUCIFERASE AND ((FUSION OR CHIMER?) AND PROTEIN)
S3	25	S2 AND PLANT

? s s2 and algae

913	S2
182584	ALGAE

S4	5	S2 AND ALGAE
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? t s4/7/1-5

4/7/1

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18613111 BIOSIS NO.: 200510307611

A cyclophilin from *Griffithsia japonica* has thermoprotective activity and is affected by CsA

AUTHOR: Cho Eun Kyung (Reprint); Lee Yoo Kyung; Hong Choo Bong

AUTHOR ADDRESS: Seoul Natl Univ, Inst Mol Biol and Genet, Seoul 151742, South Korea**South Korea

AUTHOR E-MAIL ADDRESS: choeun@snu.ac.kr

JOURNAL: Molecules and Cells 20 (1): p142-150 AUG 31 2005 2005

ISSN: 1016-8478

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Members of the multifunctional Cyp family have been isolated from a wide range of organisms. However, few functional studies have been performed on the role of these proteins as chaperones in red alga. For

studying the function of cDNA GjCyp-1 isolated from the red alga (*Griffithsia japonica*), we expressed and purified a recombinant GjCyp-1 containing a hexahistidine tag at the amino-terminus in *Escherichia coli*. An expressed **fusion protein**, H(6)GjCyp-1 maintained the stability of *E. coli* proteins up to 50 degrees C. For a functional bioassay for recombinant H6GjCyp-1, the viability of *E. coli* cells overexpressing H(6)GjCyp-1 was compared with that of cells not expressing H(6)GjCyp-1 at 50 degrees C. After high temperature treatment for 1 h, *E. coli* overexpressing H(6)GjCyp-1 survived about three times longer than *E. coli* lacking H6GjCyp-1. Measurement of the light scattering of **luciferase** (luc) showed that GjCyp-1 prevents the aggregation of luc during mild heat stress and that the thermoprotective activity of GjCyp-1 is blocked by cyclosporin A (CsA), an inhibitor of Cyps. Furthermore, the Cyp-CsA complex inhibited the growth of *E. coli* under normal conditions. The results of the GjCyp-1 bioassays as well as in vitro studies strongly suggest that Cyp confers thermotolerance to *E. coli*.

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18230415 BIOSIS NO.: 200500137052

Monitoring dynamic expression of nuclear genes in *Chlamydomonas reinhardtii* by using a synthetic **luciferase** reporter gene

AUTHOR: Fuhrmann Markus (Reprint); Hausherr Amparo; Ferbitz Lars; Schoedl Thomas; Heitzer Markus; Hegemann Peter

AUTHOR ADDRESS: Div BiotechnolCtr Excellence Fluorescent Bioanal, Univ Regensburg, D-93053, Regensburg, Germany**Germany

AUTHOR E-MAIL ADDRESS: markus.fuhrmann@vkl.uni-regensburg.de

JOURNAL: Plant Molecular Biology 55 (6): p869-881 August 2004 2004

MEDIUM: print

ISSN: 0167-4412 (ISSN print)

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: For monitoring the expression profile of selected nuclear genes in *Chlamydomonas reinhardtii* in response to altered environmental parameters or during cell cycle, in the past many RNA or **protein** samples had to be taken and analyzed by RNA hybridization or **protein** immunoblotting. Here we report the synthesis of a gene that codes for the **luciferase** of *Renilla reniformis* (RLuc) and is adapted to the nuclear codon usage of *C. reinhardtii*. This *crluc* gene was expressed alone or as a **fusion** to the zeocin resistance gene *ble* under control of different promoter variants. **Luciferase** activity was monitored in living cells, increased with the promoter strength and paralleled the amount of expressed **protein**. Under control of the *Lhcb-1* promoter the *Luc*-activity in synchronized cultures was dependent on the dark-light cycle. Additionally, *crluc* was placed under control of the *Chop-2* promoter and activity was measured under different light conditions. *Chop-2* promoter activity was found to be most pronounced under low-light and dark conditions, further supporting that channelrhodopsin-2 is most active in dark-adapted cells. We conclude that *crluc* is a reliable tool for convenient monitoring of nuclear gene expression in *C. reinhardtii*.

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17745386 BIOSIS NO.: 200400116143

Development of a **luciferase** reporter gene, luxCt, for Chlamydomonas reinhardtii chloroplast.

AUTHOR: Mayfield Stephen P (Reprint); Schultz Jason

AUTHOR ADDRESS: Department of Cell Biology and The Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 N. Torrey Pines Road, La Jolla, CA, 92037, USA**USA

AUTHOR E-MAIL ADDRESS: mayfield@scripps.edu

JOURNAL: Plant Journal 37 (3): p449-458 February 2004 2004

MEDIUM: print

ISSN: 0960-7412 (ISSN print)

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: **Luciferase** reporter genes have been successfully used in a variety of organisms to examine gene expression in living cells, but are yet to be successfully developed for use in chloroplast. Green fluorescent **protein** (gfp) has been used as a reporter of chloroplast gene expression, but because of high auto-fluorescence, very high levels of GFP accumulation are required for visualization in vivo. We have developed a **luciferase** reporter for chloroplast by synthesizing the two-subunit bacterial **luciferase** (lux)AB, as a single **fusion** **protein** in Chlamydomonas reinhardtii chloroplast codon bias. We expressed a chloroplast **luciferase** gene, luxCt, in C. reinhardtii chloroplasts under the control of the ATPase alpha subunit (atpA) or psbA promoter and 5' untranslated regions (UTRs) and the rubisco large subunit (rbcL) 3' UTR. We show that luxCt is a sensitive reporter of chloroplast gene expression, and that **luciferase** activity can be measured in vivo using a charge coupled device (CCD) camera or in vitro using a luminometer. We further demonstrate that luxCt **protein** accumulation, as measured by Western blot analysis, is proportional to luminescence, as determined both in vivo and in vitro, and that luxCt is capable of reporting changes in chloroplast gene expression during a dark to light shift. These data demonstrate the utility of the luxCt gene as a versatile and sensitive reporter of chloroplast gene expression in living cells.

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16588693 BIOSIS NO.: 200200182204

Imaging of light emission from the expression of luciferases in living cells and organisms: A review

AUTHOR: Greer Lee F; Szalay Aladar A (Reprint)

AUTHOR ADDRESS: Department of Biochemistry, School of Medicine and Department of Natural Sciences-Biology Section, Loma Linda University, Loma Linda, CA, 92354, USA**USA

JOURNAL: Luminescence (Chichester) 17 (1): p43-74 January-February, 2002

2002

MEDIUM: print

ISSN: 1522-7235

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Luciferases are enzymes that emit light in the presence of oxygen and a substrate (luciferin) and which have been used for real-time, low-light imaging of gene expression in cell cultures, individual cells, whole organisms, and transgenic organisms. Such luciferin-
%%luciferase%% systems include, among others, the bacterial lux genes of terrestrial *Photobacterium luminescens* and marine *Vibrio harveyi* bacteria, as well as eukaryotic %%luciferase%% luc and ruc genes from firefly species (*Photinus*) and the sea pansy (*Renilla reniformis*), respectively. In various vectors and in %%fusion%% constructs with other gene products such as green fluorescence %%protein%% (GFP; from the jellyfish *Aequorea*), luciferases have served as reporters in a number of promoter search and targeted gene expression experiments over the last two decades. %%Luciferase%% imaging has also been used to trace bacterial and viral infection in vivo and to visualize the proliferation of tumour cells in animal models.

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15974330 BIOSIS NO.: 200100146169

N-terminal intramolecularly conserved histidines of three domains in *Gonyaulax* %%luciferase%% are responsible for loss of activity in the alkaline region

AUTHOR: Li Liming; Liu Liyun; Hong Robert; Robertson Deborah; Hastings J Woodland (Reprint)

AUTHOR ADDRESS: Department of Molecular and Cellular Biology, Harvard University, 16 Divinity Avenue, Cambridge, MA, 02138-2020, USA**USA

JOURNAL: Biochemistry 40 (6): p1844-1849 February 13, 2001 2001

MEDIUM: print

ISSN: 0006-2960

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: *Gonyaulax* %%luciferase%% is a single-chain (approx 137 kDa) polypeptide comprising 111 N-terminal amino acids followed by three contiguous homologous domains (377 amino acids each). Each domain has %%luciferase%% activity, accounting for the earlier observation that proteolytic fragments (approx 35 kDa) of %%luciferase%% are active. The activity of the full-length native enzyme is maximal at pH 6.3, dropping to near zero at pH 8; the activity of fragments also peaks at pH 6.3 but remains high at 8. While the activity loss at higher pH might be thought to be associated with the conformation of the full-length %%protein%%, we show here that this is a property of individual domains. The three intramolecularly homologous domains, separately cloned and expressed in *Escherichia coli* as %%fusion%% proteins, exhibit pH-activity curves similar to that of the full-length enzyme. For each domain the removal of approximately 50 N-terminal amino acids resulted in an increase in the

ratio of %luciferase% activity at pH 8 relative to that at pH 6.3, such that their pH-activity profiles mimicked that of the proteolytic fragments reported earlier. Replacement of N-terminal histidines by alanine by site-directed mutagenesis identified four that are involved in the loss of activity at high pH. This system illustrates an unusual, possibly unique mechanism for pH regulation of enzyme activity, which has been postulated to be responsible for the control of the characteristic flashes of bioluminescence.

? s s2 and animal

913 S2
890279 ANIMAL

S5 53 S2 AND ANIMAL

? s s2 and (transgenic()animal)

913 S2
81855 TRANSGENIC
890279 ANIMAL
2512 TRANSGENIC(W)ANIMAL

S6 0 S2 AND (TRANSGENIC()ANIMAL)

? s s2 and transgenic

913 S2
81855 TRANSGENIC

S7 54 S2 AND TRANSGENIC

? s s7 not s5

54 S7
53 S5

S8 49 S7 NOT S5

? t s5/7/1-53

5/7/1

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0019461801 BIOSIS NO.: 200700121542

Angiotensin II type 1 receptor 1166 polymorphism A --> C increases mRNA stability and steady-state levels

AUTHOR: Lehtonen Jukka (Reprint); Paukku Kirsi; Daviet Laurent; Kontula Kimmo

AUTHOR ADDRESS: Univ Helsinki, Cent Hosp, FIN-00014 Helsinki, Finland**
Finland

JOURNAL: Circulation 114 (18, Suppl. S): p190 OCT 31 2006 2006

CONFERENCE/MEETING: 79th Annual Scientific Session of the
American-Heart-Association Chicago, IL, USA November 12 -15, 2006;
20061112

SPONSOR: Amer Heart Assoc

ISSN: 0009-7322

DOCUMENT TYPE: Meeting; Meeting Abstract

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Angiotensin II-type 1 receptor (AT1R) regulation plays an important role in the pathogenesis of hypertension and heart failure. Posttranscriptional regulation of AT1R is largely mediated by its 3'untranslated region (3'UTR). In order to analyze structure-function relationship of the 3'UTR, a number of AT1R 3'UTR constructs were created and tested. Removal of 3'UTR from AT1R message increases mRNA levels by increasing its half-life, Transfer of the 3'UTR of AT1R to 3'end of either bcl-2 or %luciferase% coding region resulted in a decrease in

the steady-state mRNA expression of these **fusion** constructs and therefore 3'UTR contains all the necessary elements for its inhibitory effect. Functional characterization of 3'UTR deletions and domain swapping constructs showed that 3'UTR contains both stabilizing and destabilizing elements resulting in increased or decreased steady-state mRNA levels. In order to identify functionally active regions, AT1R 3'UTR was subjected to random mutagenesis, After several rounds of screening, two single base alterations were found to have an effect on mRNA levels. Alteration in the base at 1255 resulted in a 3-fold decrease and at 1166 2.5-fold increase in mRNA levels. Thus AT1R 3'UTR 1166 A -> C polymorphism results in a partial loss of 3'UTR effect on mRNA destabilization. Interestingly, the carrier status of this single polymorphism located in the 3'UTR of AT1R has been associated with increased incidence of hypertension and higher rate of restenosis. In order to study the RNA-**protein** interactions, we performed RNA-shift and Northwestern assays using 3'UTR 1081-1181 as a probe and found at least three RNA-interacting proteins at sizes of 17 kDa, 35 kDa, and 45 kDa. It is tempting to speculate that changes in RNA-**protein** interactions could explain the effects of 1166 A -> C polymorphism on mRNA levels.

5/7/2

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19305177 BIOSIS NO.: 200600650572

Expression of recombinant **protein** in cho and HeLa cells and its follow-up using EGF reporter gene

BOOK TITLE: **Animal** Cell Technology: Basic & Applied Aspects, Vol 14

AUTHOR: Hendrick V (Reprint); Ribeiro de Sousa D; dos Santos Pedregal A R; Bassens C; Rigaux P; Sato K; Kotarsky K; Werenne J

BOOK AUTHOR/EDITOR: Iijima S (Editor); Nishijima KI (Editor)

AUTHOR ADDRESS: Univ Libre Bruxelles, Fac Sci, Dept Bioengn, Lab Anim Cell Biotechnol, Brussels, Belgium**Belgium

p55-59 2006

BOOK PUBLISHER: SPRINGER, PO BOX 17, 3300 AA DORDRECHT, NETHERLANDS

CONFERENCE/MEETING: 17th Annual Meeting of the

Japanese-Association-for-Animal-Cell-Technology Nagoya, JAPAN November 15-18, 2004; 20041115

SPONSOR: Japanese Assoc Anim Cell Technol

ISBN: 1-4020-4312-0 (H)

DOCUMENT TYPE: Book Chapter; Meeting

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: The goal of this paper was to evaluate the potentialities of using **chimeric** EGFP-Photinus **Luciferase** reporter gene to follow the expression of the **fusion** **protein** by following the fluorescence for the development of recombinant **protein** production process and to evaluate the conditions in which the use of butyrate stimulation of **protein** production is effective.

5/7/3

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18840450 BIOSIS NO.: 200600185845

The telomerase template antagonist GRN163L in combination with chemotherapeutics reduces tumor volume in multiple myeloma xenograft models.

AUTHOR: Ertem Server A (Reprint); Wu Kaida; Ponomarev Vladimir; Chin Allison C; Moore Malcolm A S

AUTHOR ADDRESS: Mem Sloan Kettering Canc Ctr, Moore Lab, Cell Biol Program, New York, NY 10021 USA**USA

JOURNAL: Blood 106 (11, Part 1): p971A NOV 16 2005 2005

CONFERENCE/MEETING: 47th Annual Meeting of the American-Society-of-Hematology Atlanta, GA, USA December 10 -13, 2005; 20051210

SPONSOR: Amer Soc Hematol

ISSN: 0006-4971

DOCUMENT TYPE: Meeting; Meeting Poster

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Primary hematopoietic malignancies are characterized by short telomeres, suggesting that they would be acutely susceptible to telomerase inhibition. We have previously shown that treatment with GRN163L, a lipid-conjugated 13-mer thio-phosphoramidate oligonucleotide (Geron Corporation), inhibits the growth of human multiple myeloma (CAG) and ovarian carcinoma (OVCAR3) cell lines in vitro and in vivo (AACR 2005 Annual Meeting). The CAG multiple myeloma cell line (telomere length 2.7 Kb) was transfected with a retroviral vector encoding a triple **%%fusion%%** gene for HSV-TK, firefly **%%luciferase%%** and green fluorescence **%%protein%%**, and whole **%%animal%%** bioluminescence imaging was undertaken in either a subcutaneous or disseminated NOD/SCID model with extensive spinal cord and bone marrow tumor infiltration. In a subcutaneous model with CAG, GRN163L was administered three times per week (tiw) at 36 mg/kg for 4 weeks alone or in combination with either a single dose of melphalan (5 mg/kg) or 9 doses of Velcade (0.25 mg/kg tiw). Tumor mass was reduced 56% with GRN 163L alone ($p < 0.001$), 60% with inelphalan alone ($p < 0.001$), 89% with the combination of GRN163L and melphalan ($p < 0.001$). At the dose used, Velcade alone showed no efficacy, but the combination of GRN 163L with Velcade reduced the tumor mass by 68% ($p < 0.001$) compared to controls. Data on the treatment with a mismatch control oligonucleotide in addition to dose optimization of GRN163L in combination with Melphalan and Velcade in subcutaneous and disseminated myeloma models (CAG, MM.1S) will be presented. Geron initiated a Phase I/II trial with GRN163L in chronic lymphocytic leukemia in July 2005. These data support the development of GRN 163L as an effective and safe agent for therapy of hematopoietic malignancies.

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18576999 BIOSIS NO.: 200510271499

Differential regulation of alpha-granule and dense granule secretion by an actin cytoskeletal barrier.

AUTHOR: Flaumenhaft Robert (Reprint); Dilks James R; Rozenvayn Nataliya; Monahan-Earley Rita A; Feng Dian; Dvorak Ann M

AUTHOR ADDRESS: Harvard Univ, Sch Med, Beth Israel Deaconess Med Ctr, Dept

Med, Boston, MA USA**USA
JOURNAL: Blood 104 (11, Part 1): p960A-961A NOV 16 2004 2004
CONFERENCE/MEETING: 46th Annual Meeting of the
American-Society-of-Hematology San Diego, CA, USA December 04 -07, 2004;
20041204
SPONSOR: Amer Soc Hematol
ISSN: 0006-4971
DOCUMENT TYPE: Meeting; Meeting Poster
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Platelet granule secretion is an essential component of normal arterial thrombus formation. Stimulation of platelets with strong agonists results in centralization of cytoplasmic organelles and loss of granules. These observations have lead to the supposition that cytoskeletal contraction facilitates granule secretion. Yet, the influence of the actin cytoskeleton in controlling membrane ***fusion*** events required for granule secretion remains largely unknown. Initial studies using electron microscopy revealed that the actin disrupting agents latrunculin A (4 μ M) or cytochalasin E (4 μ M) prevented pseudopod formation and granule centralization in platelets exposed to SFLLRN or PMA, but did not prevent degranulation. We next determined the effects of disruption of the actin cytoskeleton on α -granule secretion by monitoring P-selectin expression and β -thromboglobulin release. Incubation of platelets with either latrunculin A or cytochalasin E failed to stimulate α -granule secretion, but increased the rate of SFLLRN-induced α -granule secretion by 3.5-fold. Cytoskeletal disruption also augmented the degree of SFLLRN-induced α -granule secretion by 41 \pm 18% and reduced the amount of SFLLRN required to cause half-maximal stimulation by 2-fold. Incubation with latrunculin A stimulated α -granule secretion by the weak secretagogues epinephrine or ADP by 7.6-fold and 5.4-fold, respectively. Cytoskeletal disruption also facilitated β -thromboglobulin release in response to SFLLRN, epinephrine, or ADP. In platelets permeabilized in the absence of ATP, exposure to 2 μ M latrunculin A resulted in a 6.5- and 3.5-fold increase in α -granule release induced by Ca^{2+} - or GTP- γ -S, respectively. Antibodies directed at a SNARE ***protein*** termed vesicle-associated ***fusion*** ***protein*** (VAMP) inhibited latrunculin A-dependent α -granule secretion. Thus, disruption of the actin cytoskeletal barrier by latrunculin A supports SNARE ***protein***-dependent membrane ***fusion***. Since actin acts as a barrier to α -granule secretion, we evaluated α -granules purified by subcellular fractionation for the presence of F-actin. Purified α -granules, but not phospholipid micelles, bound the F-actin probe FITC-phalloidin as determined by flow cytometry. FITC-phalloidin binding was inhibited in a dose-dependent manner by latrunculin A. These data indicated that α -granules are coated with F-actin that could serve a barrier function. We next evaluated the effects of cytoskeletal disruption on dense granule secretion by monitoring ADP/ATP release using a luciferin-***luciferase*** based assay and by quantifying [^3H]serotonin release. Cytoskeletal disruption by 4 μ M latrunculin A failed to affect the degree of dense granule secretion from platelets stimulated by either SFLLRN, epinephrine, or ADP. Yet, 200 μ M latrunculin A stimulated substantial dense granule release in the absence of agonist exposure and augmented SFLLRN-induced dense granule release by 2-fold. In contrast, 200 μ M latrunculin A abolished SFLLRN-induced α -granule secretion. These observations indicate that the

cytoskeleton differentially regulates alpha-granule and dense granule secretion. Our results also suggest that while some degree of actin polymerization is required for alpha-granule secretion, dense granule secretion is not dependent on actin polymerization.

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18572409 BIOSIS NO.: 200510266909

High efficiency transduction of human mesenchymal stem cells using retroviral gene transfer with triple reporter genes.

AUTHOR: Niu Ting (Reprint); Najjar Amer M; Robinson Simon N; Yang Hong; Decker William K; McMannis John D; Ng Jingjing; Thomas Michael W; Xing Dongxia; Champlin Richard E; Gelovani Juri G; Shpall Elizabeth J

AUTHOR ADDRESS: Univ Texas, MD Anderson Canc Ctr, Houston, TX 77030 USA**
USA

JOURNAL: Blood 104 (11, Part 2): p150B NOV 16 2004 2004

CONFERENCE/MEETING: 46th Annual Meeting of the
American-Society-of-Hematology San Diego, CA, USA December 04 -07, 2004;
20041204

SPONSOR: Amer Soc Hematol

ISSN: 0006-4971

DOCUMENT TYPE: Meeting; Meeting Abstract

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Cotransplantation of ex vivo-expanded mesenchymal stem cells (MSC) together with hematopoietic stem cells hastens hematopoietic recovery in %animal% models and potentially humans. The in vivo mechanisms of migration, homing and long-term viability of MSC following implantation are not well understood. Reporter gene labeling would permit the tracking of transplanted MSC in vivo through noninvasive imaging techniques, namely fluorescence, bioluminescence, and nuclear imaging. Accordingly, a gibbon ape leukemia virus (GALV)-pseudotyped retrovirus was utilized to transfer a triple %fusion% gene reporter system, expressing enhanced green fluorescent %protein% (eGFP), %luciferase%, and herpes simplex virus type one thymidine kinase (HSV1-tk) into MSC. Human MSC were isolated and propagated from normal bone marrow aspirates by culturing ficoll-separated fractions in alpha-MEM medium supplemented with 20% fetal bovine serum. FACS analysis confirmed the MSC phenotype, with cells expressing HLA-Class I, CD105, CD73, CD90 and CD166 antigens and not expressing HLA-Class II, CD80, CD31, CD34 and CD45 antigens. No significant phenotypic changes were observed in early and late passage numbers of MSC. MSC were transduced during their exponential growth phase (as determined by growth curve measurements), with virus-containing medium (VCM) from the PG 13 packaging cells (targeting Pit-1 receptors) for 24 hours in the presence of different concentrations of the cationic polymer, polybrene, ranging from 2 μ g/ml to 12 μ g/ml. The 2 μ g/ml concentration appeared to be optimal for transduction of MSC. VCM was harvested following a 24-hour incubation of the PG13 packaging cells at 32 degrees C or 37 degrees C. These initial experiments suggested that incubation at 32 degrees C appeared to be optimal for virus production. Repeated VCM exposure at 24-hour intervals for 2 or 3 days did not increase the percentage of transduced cells as determined by flow cytometry (eGFP-positive). VCM

harvested at 32 degrees C added to MSC in the presence of 2 μ g/ml polybrene, yielded a 62% transduction efficiency following one round of exposure. U87 human glioma cells were used throughout as a positive control to verify retrovirus efficacy and demonstrated > 90% transduction efficiency. MSC clones highly expressing triple reporter genes are being selected and propagated in our laboratory to image transduced cells in NOD/SCID mice, thereby providing a better understanding of the potential role of these cells in transplantation processes.

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18255151 BIOSIS NO.: 200500161323

Spying on cancer: Molecular imaging in vivo with genetically encoded reporters

AUTHOR: Gross Shimon; Piwnica-Worms David (Reprint)

AUTHOR ADDRESS: Sch MedMallinckrodt Inst RadiolMol Imaging Ctr, Washington Univ, St Louis, MO, 63110, USA**USA

AUTHOR E-MAIL ADDRESS: piwnica-wormsd@mir.wustl.edu

JOURNAL: Cancer Cell 7 (1): p5-15 January 2005 2005

MEDIUM: print

ISSN: 1535-6108 (ISSN print)

DOCUMENT TYPE: Article; Literature Review

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Genetically encoded imaging reporters introduced into cells and transgenic animals enable noninvasive, longitudinal studies of dynamic biological processes in vivo. The most common reporters include firefly **luciferase** (bioluminescence imaging), green fluorescence **protein** (fluorescence imaging), herpes simplex virus-1 thymidine kinase (positron emission tomography), and variants with enhanced spectral and kinetic properties. When cloned into promoter/enhancer sequences or engineered into **fusion** proteins, imaging reporters allow transcriptional regulation, signal transduction, **protein**-**protein** interactions, oncogenic transformation, cell trafficking, and targeted drug action to be spatiotemporally resolved in vivo. Spying on cancer with genetically encoded imaging reporters provides insight into cancer-specific molecular machinery within the context of the whole **animal**.

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18241305 BIOSIS NO.: 200500148370

Enhanced antilymphoma efficacy of CD19-redirected influenza MPl-specific CTLs by cotransfer of T cells modified to present influenza MPl

AUTHOR: Cooper Laurence J N (Reprint); Al-Kadhimi Zaid; Serrano Lisa Marie; Pfeiffer Timothy; Olivares Simon; Castro Adrian; Chang Wen-Chung;

Gonzalez Sergio; Smith David; Forman Stephen J; Jensen Michael C

AUTHOR ADDRESS: Beckman Res InstDiv Mol MedDiv Pediat Hematol Oncol, City Hope Natl Med Ctr, 1500 E Duarte Rd, Duarte, CA, 91010, USA**USA

AUTHOR E-MAIL ADDRESS: lcooper@coh.org

JOURNAL: Blood 105 (4): p1622-1631 February 15, 2005 2005
MEDIUM: print
ISSN: 0006-4971
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: To enhance the in vivo antitumor activity of adoptively transferred, CD19-specific **chimeric** antigen receptor (CAR)-redirected cytotoxic T lymphocytes (CTLs), we studied the effect of restimulating CAR+ CTLs through their endogenous virus-specific T-cell antigen receptor (TcR) by the cotransfer of engineered T-cell antigen-presenting cells (T-APCs). Using influenza A matrix **protein** 1 (MP1) as a model antigen, we show that ex vivo-expanded CD4+ and CD8+ T-APCs expressing a hygromycin phosphotransferase-MP1 **fusion** **protein** (HyMP1) process and present MP1 to autologous human leukocyte antigen (HLA)-restricted, MP1-specific CD4+ and CD8+ CTL precursors. The MP1-specific CTLs are amenable to subsequent genetic modification to express a CD19-specific CAR, designated CD19R, and acquire HLA-unrestricted reactivity toward CD19+ leukemia and lymphoma tumor targets while maintaining HLA-restricted MP1 specificity. The restimulation of MP1 x CD19 dual-specific CTLs in vivo by the adoptive transfer of irradiated HyMP1+ T-APCs resulted in the enhanced antilymphoma potency of bispecific effector cells, as measured by elimination of the biophotonic signal of established firefly **luciferase**-expressing Burkitt lymphoma xenografts in nonobese diabetic/severe combined immunodeficiency (NOD/scid) animals compared with control groups restimulated by Hy+MP1neg T-APCs. Engineered T-APCs are a novel and versatile antigen-delivery system for generating antigen-specific T cells in vitro and enhancing the in vivo effector functioning of CAR-redirected antitumor effector cells. Copyright 2005 by The American Society of Hematology.

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18211268 BIOSIS NO.: 200500117446
Initiation of **protein** synthesis by a labeled derivative of the Lactobacillus casei DN-114 001 strain during transit from the stomach to the cecum in mice harboring human microbiota
AUTHOR: Oozeer R; Mater D D G; Goupil-Feuillerat N; Corthier G (Reprint)
AUTHOR ADDRESS: Unite Ecol and Physiol Syst Digestif, INRA, F-78350, Jouy En Josas, France**France
AUTHOR E-MAIL ADDRESS: corthier@jouy.inra.fr
JOURNAL: Applied and Environmental Microbiology 70 (12): p6992-6997
December 2004 2004
MEDIUM: print
ISSN: 0099-2240 (ISSN print)
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Although studies on the survival of bacteria in the digestive tract have been reported in the literature, little data are available on the physiological adaptation of probiotics to the digestive environment.

In previous work, a transcriptional **fusion** system (i.e., **luciferase** genes under the control of a deregulated promoter) was used to demonstrate that a derivative of the *Lactobacillus casei* DN-114 001 strain, ingested in a fermented milk and thus exhibiting initially a very weak metabolic activity, synthesized proteins de novo after its transit in the digestive tract of mice harboring human microbiota (known as human-microbiota-associated mice). With the same genetic system and **animal** model, we here investigate for the first time the ability of *L. casei* to reinitiate synthesis in the different digestive tract compartments. In this study, most ingested *L. casei* cells transited from the stomach to the duodenum-jejunum within 1 h postingestion. No **luciferase** activity was observed in these digestive tract compartments after the first hour. At later times, the bulk of bacteria had transited to the ileum and the cecum. **Luciferase** synthesis was detected between 1.5 and 2.0 h postingestion at the ileal level and from 1.5 h to at least 6.0 h postingestion in the cecum, where the activity remained at a maximum level. These results demonstrate that ingested *L. casei* (derivative of the DN-114 001 strain) administered via a fermented milk has already reinitiated **protein** synthesis when it reaches the ileal and cecal compartments.

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18042515 BIOSIS NO.: 200400413304

Rapid and sensitive detection of retrovirus entry by using a novel

luciferase-based content-mixing assay

AUTHOR: Kolokol'tsov Andrey A; Davey Robert A (Reprint)

AUTHOR ADDRESS: Med BranchDept Microbiol and Immunol, Univ Texas, 301 Univ Blvd, Galveston, TX, 77555, USA**USA

AUTHOR E-MAIL ADDRESS: radavey@utmb.edu

JOURNAL: Journal of Virology 78 (10): p5124-5132 May 2004 2004

MEDIUM: print

ISSN: 0022-538X_(ISSN print)

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: We describe a novel assay that permits measurement of entry of murine leukemia virus and pseudotypes with greater sensitivity and more rapidly than previously possible. To achieve this, we encapsulated a sensitive reporter enzyme, **luciferase**, directly into fully infectious, intact viral particles. The enzyme is specifically targeted to the viral lumen, as a C-terminal **fusion** on the viral envelope **protein**. Only when the incorporated **luciferase** is released from the viral lumen and gains access to its substrates is light emitted and readily detected. When cells are perfused with luciferin, quantitative measurements of entry can be made in real time on live cells. Uniquely, the amount of cell-bound virus can be determined in the same assay by addition of detergent to expose the **luciferase**. We demonstrate that virus carrying a mutation in the **fusion** peptide binds normally to cells but is unable to infect them and gives no entry signal. Using this assay, we show that inhibitors of endosomal acidification inhibit signal from vesicular stomatitis virus pseudotypes but not murine leukemia virus, consistent with a pH-independent mode of

entry for the latter virus. Additionally, the **fusion** kinetics are rapid, with a half-life of 25 min after a delay of 10 to 15 min. The future use of this assay will permit a detailed examination of the entry mechanism of viruses and provide a convenient platform to discover novel entry inhibitors. The design also permits packaging of potential therapeutic **protein** cargoes into functional virus particles and their specific delivery to cellular targets.

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17997840 BIOSIS NO.: 200400368629

A one-step approach to obtain cell clones expressing tetracycline-responsive transactivators

AUTHOR: Munoz Iciar; Gomez Ana (Reprint); Zanuy Silvia; Carrillo Manuel

AUTHOR ADDRESS: Inst Acuicultura TorrelasalDept Fish Reprod Physiol, CSIC, Castellon, 12595, Spain**Spain

AUTHOR E-MAIL ADDRESS: ana@iats.esic.es

JOURNAL: Analytical Biochemistry 331 (1): p153-160 August 1, 2004 2004

MEDIUM: print

ISSN: 0003-2697 (ISSN print)

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Despite the wide application of the tetracycline-regulated gene expression system, several drawbacks in establishing the system in in vitro-cultured cells have been described. Most of the problems are related to the obtainment of a reliable tetracycline-regulated cell clone, which often results in arduous labor. We describe here a new approach to facilitate the screening and selection of such cell clones. We have constructed a tetracycline-responsive plasmid that harbors an antibiotic resistance gene fused to the enhanced green fluorescent **protein** (EGFP) gene and the **luciferase** gene, both under the control of a bidirectional promoter. We demonstrate that the selection of tetracycline-regulated clones is highly simplified by using this plasmid. Only clones expressing the system in a functional manner are able to survive under antibiotic selection. In addition, a quick characterization of the responsiveness of the clones is possible by monitoring GFP expression in vivo. Copyright 2004 Elsevier Inc. All rights reserved.

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17974242 BIOSIS NO.: 200400345031

Quantification of virus-envelope-mediated cell **fusion** using a tetracycline transcriptional transactivator: **fusion** does not correlate with syncytium formation

AUTHOR: Ou Wu; Xiong Ying; Silver Jonathan (Reprint)

AUTHOR ADDRESS: Mol Microbiol LabNIH, NIAID, Bldg 4, Room 336, 4 Ctr Dr, MSC 0460, Bethesda, MD, 20892, USA**USA

AUTHOR E-MAIL ADDRESS: wou@niaid.nih.gov; jsilver@nih.gov

JOURNAL: Virology 324 (2): p263-272 July 1, 2004 2004

MEDIUM: print
ISSN: 0042-6822 _(ISSN print)
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Cell %%%fusion%% occurs in many cellular processes and viral infections. We developed a new, quantitative cell %%%fusion%% assay based on the tetracycline-controlled transactivator (tTA)-induced expression of a %%luciferase%% reporter gene. The assay is objective, sensitive, linear over 2-3 orders of magnitude, amenable to microtiter-plate format, and generalizable to study %%%fusion%% mediated by a variety of genes. Applied to HIV and MLV, cell %%%fusion%% paralleled virus entry in terms of co-receptor requirements, need for post-translational processing of envelope, and complementation of SU mutations by soluble receptor-binding domain. However, biochemically measured %%%fusion%% did not correlate with syncytia detected by standard light microscopy. When the assay indicated cell.%%fusion%% occurred but overt syncytia were not observed, confocal microscopy using fluorescent %%protein%% markers showed that %%%fusion%% was limited mainly to pairs of cells. Such nonprogressive cell %%%fusion%% suggests that post-translational processing of envelope may be altered in heterokaryons co-expressing envelope and receptor. Published by Elsevier Inc.

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17948074 BIOSIS NO.: 200400318831
Molecular imaging of homodimeric %%protein%%-%%protein%% interactions in living subjects
AUTHOR: Massoud Tarik F; Paulmurugan Ramasamy; Gambhir Sanjiv S (Reprint)
AUTHOR ADDRESS: Sch MedJames H Clark CtrDept Radiol, Stanford Univ, 318 Campus Dr, East Wing, 1st Floor, Stanford, CA, 94305, USA**USA
AUTHOR E-MAIL ADDRESS: sgambhir@stanford.edu
JOURNAL: FASEB Journal 18 (7): May 2004 2004
MEDIUM: print
ISSN: 0892-6638 _(ISSN print)
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Homodimeric %%protein%% interactions are potent regulators of cellular functions, but are particularly challenging to study in vivo. We used a split synthetic renilla %%luciferase%% (hRLUC) complementation-based bioluminescence assay to study homodimerization of herpes simplex virus type 1 thymidine kinase (TK) in mammalian cells and in living mice. We quantified and imaged homodimerization of TK %%chimeras%% containing N-terminal (N-hRLUC) or C-terminal (C-hRLUC) fragments of hRLUC in the upstream and downstream positions, respectively (tail-to-head homodimer). This was monitored using luminometry (68-fold increase, and was significantly (P 0.01) above background light emission) and by CCD camera imaging of living mice implanted with ex vivo transfected 293T cells (2.7-fold increase, and is significantly (P 0.01) above background light emission). We also made a mutant-TK to generate

N-hRLUC mutant TK and mutant TK-C-hRLUC by changing a single amino acid at position 318 from arginine to cysteine, a key site that has previously been reported to be essential for TK homodimerization, to support the specificity of the hRLUC complementation signal from TK homodimerization. Ex vivo substrate (8-3H Penciclovir) accumulation assays in 293T cells expressing the TK **protein** **chimeras** showed active TK enzyme. We also devised an experimental strategy by constructing variant TK **chimeras** (possessing extra N-hRLUC or C-hRLUC 'spacers') to monitor incremental lack of association of the tail-to-head TK homodimer. Application of this potentially generalizable assay to screen for molecules that promote or disrupt ubiquitous homodimeric **protein** - **protein** interactions could serve not only as an invaluable tool to understand biological networks but could also be applied to drug discovery and validation in living subjects.

5/7/13

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17903572 BIOSIS NO.: 200400274329

PERIOD2::**LUCIFERASE** real-time reporting of circadian dynamics reveals persistent circadian oscillations in mouse peripheral tissues

AUTHOR: Yoo Seung-Hee; Yamazaki Shin; Lowrey Phillip L; Shironomura Kazuhiro ; Ko Caroline H; Buhr Ethan D; Siepkha Sandra M; Hong Hee-Kyung; Oh Won Jun; Yoo Ook Joon; Menaker Michael; Takahashi Joseph S (Reprint)

AUTHOR ADDRESS: Howard Hughes Med Inst, Northwestern Univ, 2205 Tech Dr, Evanston, IL, 60208, USA**USA

AUTHOR E-MAIL ADDRESS: j-takahashi@northwestern.edu

JOURNAL: Proceedings of the National Academy of Sciences of the United States of America 101 (15): p5339-5346 April 13, 2004 2004

MEDIUM: print

ISSN: 0027-8424 (ISSN print)

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Mammalian circadian rhythms are regulated by the suprachiasmatic nucleus (SCN), and current dogma holds that the SCN is required for the expression of circadian rhythms in peripheral tissues. Using a PERIOD2::**LUCIFERASE** **fusion** **protein** as a real-time reporter of circadian dynamics in mice, we report that, contrary to previous work, peripheral tissues are capable of self-sustained circadian oscillations for >20 cycles in isolation. In addition, peripheral organs expressed tissue-specific differences in circadian period and phase. Surprisingly, lesions of the SCN in mPer2Luciferase knockin mice did not abolish circadian rhythms in peripheral tissues, but instead caused phase desynchrony among the tissues of individual animals and from **animal** to **animal**. These results demonstrate that peripheral tissues express self-sustained, rather than damped, circadian oscillations and suggest the existence of organ-specific synchronizers of circadian rhythms at the cell and tissue level.

5/7/14

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17747502 BIOSIS NO.: 200400118259

Regulation of laminin expression in mesangial cells by high glucose, glucosamine and IGF-1.

AUTHOR: Singh Lalit P (Reprint); Crook Errol D

AUTHOR ADDRESS: The John D. Dingell VA Medical Center, Wayne State

University School of Medicine, Detroit, MI, USA**USA

JOURNAL: Journal of the American Society of Nephrology 14 (Abstracts Issue): p598A-599A November 2003 2003

MEDIUM: print

CONFERENCE/MEETING: Meeting of the American Society of Nephrology Renal Week San Diego, CA, USA November 12-17, 2003; 20031112

SPONSOR: American Society of Nephrology

ISSN: 1046-6673

DOCUMENT TYPE: Meeting; Meeting Abstract; Meeting Poster

RECORD TYPE: Citation

LANGUAGE: English

5/7/15

DIALOG(R)File 5:Biosis Previews(R)

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17692435 BIOSIS NO.: 200400063192

p300 and serum response factor are involved in Rho kinase-mediated c-fos gene expression in cardiac myocytes.

AUTHOR: Sakoda Tsuyoshi (Reprint); Naka Toshio (Reprint); Abe Takashi

(Reprint); Fujioka Yoshio (Reprint); Tsujino Takeshi (Reprint); Iso

Tatsuya; Hamamori Yasuo; Ohyanagi Mitsumasa (Reprint)

AUTHOR ADDRESS: Dept of Internal Medicine, Cardiovascular Div, Hygo College of Medicine, Nishinomiya, Japan**Japan

JOURNAL: Circulation 108 (17 Supplement): pIV-116 October 28, 2003 2003

MEDIUM: print

CONFERENCE/MEETING: American Heart Association Scientific Sessions 2003 Orlando, FL, USA November 09-12, 2003; 20031109

SPONSOR: American Heart Association

ISSN: 0009-7322 (ISSN print)

DOCUMENT TYPE: Meeting; Meeting Abstract

RECORD TYPE: Citation

LANGUAGE: English

5/7/16

DIALOG(R)File 5:Biosis Previews(R)

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17636083 BIOSIS NO.: 200400006840

JUMONJI, a critical factor for cardiac development, functions as a transcriptional repressor.

AUTHOR: Kim Tae-Gyun; Kraus Jonathan C; Chen Junqin; Lee Youngsook (Reprint)

AUTHOR ADDRESS: Dept. of Anatomy, Medical School, University of Wisconsin, 1300 University Ave., Madison, WI, 53706, USA**USA

AUTHOR E-MAIL ADDRESS: youngsooklee@facstaff.wisc.edu

JOURNAL: Journal of Biological Chemistry 278 (43): p42247-42255 October 24, 2003 2003

MEDIUM: print

ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: JUMONJI (JMJ) is a nuclear factor that is critical for normal cardiovascular development, evidenced by the analysis of jmj homozygous mutant mice. However, the molecular function of JMJ remains to be elucidated. In the present study, we investigated whether JMJ is a transcriptional modulator. Reporter gene assays using the GAL4-DNA binding domain fused to JMJ and a reporter gene consisting of the GAL4 binding sites upstream of a **luciferase** reporter gene indicated that JMJ functions as a powerful transcriptional repressor. The DNA binding motif of JMJ was determined using CASTing experiments by incubating a random oligonucleotide library with the GST-JMJ **fusion protein** coupled to agarose beads. Among the selected binding oligonucleotides, the high affinity DNA binding sequences were identified by gel retardation assays. JMJ repressed expression of the reporter genes containing the high affinity JMJ binding sequences, indicating that JMJ is a DNA-binding transcriptional repressor. The domains for transcriptional repression, DNA binding, and nuclear localization signal were mapped by mutational analyses using reporter gene assays, gel retardation assays, and immunostaining experiments, respectively. The present data demonstrate for the first time that JMJ functions as a DNA-binding transcriptional repressor. Therefore, JMJ may play a critical role in transcription factor cascade to regulate expression of heart-specific genes and normal cardiac development.

5/7/17
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17634404 BIOSIS NO.: 200400005161
c-Jun N-terminal kinases (JNK) antagonize cardiac growth through cross-talk with calcineurin-NFAT signaling.
AUTHOR: Liang Qiangrong; Bueno Orlando F; Wilkins Benjamin J; Kuan Chia-Yi; Xia Ying; Molkentin Jeffery D (Reprint)
AUTHOR ADDRESS: Department of Pediatrics, Children's Hospital Medical Center, University of Cincinnati, 3333 Burnet Avenue, Cincinnati, OH, 45229-3039, USA**USA
AUTHOR E-MAIL ADDRESS: jeff.molkentin@chmcc.org
JOURNAL: EMBO (European Molecular Biology Organization) Journal 22 (19): p 5079-5089 October 1, 2003 2003
MEDIUM: print
ISSN: 0261-4189 (ISSN print)
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The c-Jun N-terminal kinase (JNK) branch of the mitogen-activated **protein kinase** (MAPK) signaling pathway regulates cellular differentiation, stress responsiveness and apoptosis in multicellular eukaryotic organisms. Here we investigated the functional importance of JNK signaling in regulating differentiated cellular growth in the post-mitotic myocardium. JNK1/2 gene-targeted mice and transgenic mice expressing dominant negative JNK1/2 were determined to have enhanced

myocardial growth following stress stimulation or with normal aging. A mechanism underlying this effect was suggested by the observation that JNK directly regulated nuclear factor of activated T-cell (NFAT) activation in culture and in transgenic mice containing an NFAT-dependent **luciferase** reporter. Moreover, calcineurin Abeta gene targeting abrogated the pro-growth effects associated with JNK inhibition in the heart, while expression of an MKK7-JNK1 **fusion** **protein** in the heart partially reduced calcineurin-mediated cardiac hypertrophy. Collectively, these results indicate that JNK signaling antagonizes the differentiated growth response of the myocardium through direct cross-talk with the calcineurin-NFAT pathway. These results also suggest that myocardial JNK activation is primarily dedicated to modulating calcineurin-NFAT signaling in the regulation of differentiated heart growth.

5/7/18

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17459946 BIOSIS NO.: 200300415608

Effect of lipid compositions on gene transfer into 293 cells using sendai F/HN-virosomes.

AUTHOR: Kim Hong Sung; Park Yong Serk (Reprint)

AUTHOR ADDRESS: Department of Biomedical Laboratory Science, College of Health Science, Yonsei University, Wonju, 220-710, South Korea**South Korea

AUTHOR E-MAIL ADDRESS: yspark@dragon.yonsei.ac.kr

JOURNAL: Journal of Biochemistry and Molecular Biology 35 (5): p459-464
September 30, 2003 2003

MEDIUM: print

ISSN: 1225-8687

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Fusogenic liposomes that incorporate Sendai virus envelope proteins, so-called Sendai virosomes, have been developed for in vitro and in vivo genetic modification of **animal** cells. In this study, several different virosomes of varying lipid compositions were formulated and their in vitro gene-transfer efficiencies compared. The virosomes were prepared by quantitative reconstitution of the Sendai envelope, **fusion** (F) and hemagglutinin-neuraminidase (HN) proteins into liposomal vesicles. Virosomes that contained **luciferase** reporter genes were tested in 293 transformed human kidney cells. F/HN-virosomes that were prepared with an artificial Sendai viral envelope (ASVE-virosomes) or phosphatidylserine (PS-virosomes) exhibited an 8- or 6-fold higher gene-transfer efficiency than cationic liposomes that were made with 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP). F/HN-virosomes that were prepared with phosphatidic acid (PA-virosomes) instead of PS were less efficient in gene transfer than either ASVE- or PS-virosomes. In addition, the gene-transfer capability of ASVE- and PS-virosomes was maximal at a Ca²⁺ concentration of 510 mM. These results suggest that the incorporated lipid components significantly affect the in vitro gene transfer that is mediated by Sendai F/HN-virosomes.

5/7/19
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17409109 BIOSIS NO.: 200300367828
AML1/MTG8 Leukemic %Protein% Induces Activation of STAT3. .
AUTHOR: Aoki Kenichi (Reprint); Shimoda Kazuya (Reprint); Matsuda Tadashi (Reprint); Numata Akihiko (Reprint); Kamezaki Kenjiro (Reprint); Takase Ken (Reprint); Haro Takashi (Reprint); Miyamoto Toshihiro (Reprint); Nagafuji Koji (Reprint); Gondo Hisashi (Reprint); Harada Mine (Reprint)
AUTHOR ADDRESS: Medicine and Biosystemic Science, Kyushu University Graduate School of Medical Sciences, Fukuoka, Fukuoka, Japan**Japan
JOURNAL: Blood 100 (11): pAbstract No. 4344 November 16, 2002 2002
MEDIUM: print
CONFERENCE/MEETING: 44th Annual Meeting of the American Society of Hematology Philadelphia, PA, USA December 06-10, 2002; 20021206
SPONSOR: American Society of Hematology
ISSN: 0006-4971
DOCUMENT TYPE: Meeting; Meeting Poster; Meeting Abstract
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The reciprocal translocation t(8;21)(q22;q22) is one of the most frequent chromosomal aberrations in acute myelogenous leukemia (AML), and this chromosomal translocation present in approximately 40% to 50% of patient with M2 type AML. At the molecular level, this aberration rearranges the gene for the AML1-transcription factor on chromosome 21, which is essential for normal hematopoiesis, to the MTG8 gene on chromosome 8, thereby leading to a specific AML1/MTG8 %fusion% %protein%. The recent finding that the MTG8 portion of AML1/MTG8 interacts with the histone deacetylase complex involved in transcriptional repression of target genes is consistent with the idea that AML1/MTG8 interferes with AML1-dependent transactivation. However, the mechanism of gene regulation by AML1/MTG8 varies depending on the target genes. At present, the mechanism of AML1/MTG8 responsible for leukemogenic transformation remain to be not defined. We demonstrated that AML1 %protein% and AML1/MTG8 %fusion% %protein% interacted with Stat3 (Signal transducers and activators of transcription). Since Stat3 works as an oncogene, we examined whether this interaction between AML1/MTG8 and Stat3 affected the Stat3 activity. AML1/MTG8 %fusion% %protein% enhanced the Stat3 activity in the %luciferase% assay using the promoter of APRE, although AML1 %protein% slightly decreased the Stat3 activity. Then the leukemogenesis induced by AML1/MTG8 might be in part occurred through the activation of Stat3.

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17404886 BIOSIS NO.: 200300363605
Sustained expression of Fc-%fusion% cytokine following in vivo electroporation and mouse strain differences in expression levels.
AUTHOR: Jiang Jingjing; Yamato Eiji; Miyazaki Jun-ichi (Reprint)
AUTHOR ADDRESS: Division of Stem Cell Regulation Research, Graduate School of Medicine, Osaka University, 2-2 Yamadaoka, G6, Suita, Osaka, 565-0871,

Japan**Japan
AUTHOR E-MAIL ADDRESS: jimiyaza@nutri.med.osaka-u.ac.jp
JOURNAL: Journal of Biochemistry (Tokyo) 133 (4): p423-427 Apr. 2003 2003
MEDIUM: print
ISSN: 0021-924X
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: We previously demonstrated that cytokine expression following intramuscular gene transfer of a naked plasmid is increased 2 logs by in vivo electroporation, but the relatively low expression levels of the encoded **protein** is still a limitation for successful gene therapy and gene function studies. We recently reported that the serum viral IL-10 levels achieved by electroporation-mediated intramuscular delivery of pCAGGS-vIL10, a viral IL-10-expressing plasmid, can be further enhanced by modifying the plasmid into an immunoglobulin **fusion** **protein** expression plasmid, pCAGGS-vIL10/Fc. Here we examined the applicability of this approach to the expression of an endogenous cytokine, IL-10, in two different inbred mouse strains. We obtained sustained high serum levels of IL-10 in C3H/HeJ mice (C3H), but the level and duration of the gene expression was mouse-strain dependent. Although the serum IL-10 level was also increased by using the IL-10/Fc gene plasmid in C57BL/6 mice (B6), IL-10/Fc and a **luciferase** reporter showed significantly lower levels in B6 than in C3H mice, and the persistence of pCAGGS-IL10/Fc expression ranged from several days in B6 mice to more than one month in C3H mice. These results suggest that the electroporation-mediated intramuscular delivery of the immunoglobulin **fusion** **protein** expression plasmid is simple and very efficient, but mouse strain differences in transgene expression should be taken into consideration in its use.

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17400138 BIOSIS NO.: 200300358857
PDE4 inhibitors suppress TGF-beta-stimulated MCP-1 expression by mesangial cells.
AUTHOR: Grande Joseph Peter (Reprint); Cheng Jingfei; Warner Gina M; Gray Catherine E
AUTHOR ADDRESS: Laboratory Medicine and Pathology, Mayo Clinic, 200 First Street SW, Rochester, MN, 55905, USA**USA
AUTHOR E-MAIL ADDRESS: grande.joseph@mayo.edu; cheng.jingfei@mayo.edu; warner.gina@mayo.edu; gray.catherine@mayo.edu
JOURNAL: FASEB Journal 17 (4-5): pAbstract No. 160.12 March 2003 2003
MEDIUM: e-file
CONFERENCE/MEETING: FASEB Meeting on Experimental Biology: Translating the Genome San Diego, CA, USA April 11-15, 2003; 20030411
SPONSOR: FASEB
ISSN: 0892-6638 (ISSN print)
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: MC possess functionally compartmentalized intracellular pools of

cAMP regulated by distinct isozymes of cAMP phosphodiesterases. We have previously demonstrated that PDE3 but not PDE4 inhibitors suppress MC mitogenesis, whereas PDE4 but not PDE3 inhibitors suppress reactive oxygen species generation by MC (J Biol Chem 272:9854,1997). We therefore sought to determine whether compartmentalized pools of cAMP regulate expression of MCP-1, a proinflammatory cytokine expressed in progressive renal disease. We found that TGF-beta1 induces MCP-1 mRNA (3.6 fold) and increases transcription of a **chimeric MCP-1 promoter-luciferase** construct (+24%). Rolipram (Rp, a PDE4 inhibitor) suppressed TGF-beta stimulated MCP-1 mRNA (-30%); lixazinone (a PDE3 inhibitor) was without significant effect. The PKA inhibitor 14-22 Amide reversed the inhibitory effect of Rp on MCP-1 expression; co-transfection of MC with a constitutively active PKA construct and the MCP-1 reporter decreased transcriptional activity by 47%. We conclude that MCP-1 expression in MC is regulated by a functionally compartmentalized intracellular pool of cAMP directed by PDE4. This work was supported by National Institutes of Health Grants DK16105 and DK55603.

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17361060 BIOSIS NO.: 200300319779

Conditionally immortalized cell line of inducible metanephric mesenchyme.

AUTHOR: Levashova Zoia B (Reprint); Plisov Sergei Y; Perantoni Alan O

AUTHOR ADDRESS: Laboratory of Comparative Carcinogenesis, National Cancer Institute, National Institutes of Health, Bldg. 538, Room 221, Frederick, MD, 21702-1201, USA**USA

AUTHOR E-MAIL ADDRESS: levashovaz@ncifcrf.gov

JOURNAL: Kidney International 63 (6): p2075-2087 June 2003 2003

MEDIUM: print

ISSN: 0085-2538 (ISSN print)

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Background: The mesenchymal-epithelial conversion of metanephric mesenchyme (MM) in the formation of nephronic tubules has long served as a paradigm for inductive signaling in morphogenesis. However, the mechanisms underlying this differentiation have remained an enigma due to insufficient numbers of primary mesenchymal cells that must be isolated manually from **animal** embryos. To overcome this problem, we have established a conditionally immortalized cell line, the rat-inducible metanephric mesenchyme (RIMM-18) by transfection of primary mesenchymal cells with a vector, encoding an estradiol-dependent E1A-ER **fusion protein**. Methods: Reverse transcription-polymerase chain reaction (RT-PCR), **luciferase** reporter assay, electrophoretic mobility shift assay, immunocytochemical, and immunohistochemical stainings were used to characterize the established cell line. Results: We demonstrate that in the presence of estradiol, the RIMM-18 cell line proliferates continuously, maintaining mesenchymal characteristics for over 40 passages. These cells are vimentin-positive and cytokeratin-negative. Under inductive conditions in the absence of estradiol, they are responsive to a number of cytokines, which are established inducers of mesenchymal cells in vivo and in vitro (i.e., fibroblast growth factor 2 (FGF2), leukemia inhibitory factor (LIF), and transforming growth

factor-beta2 (TGF-beta2)). We show the presence in RIMM-18 cells of specific **protein** markers and functionally active signaling pathways required for induction of tubule formation in MM. Furthermore, induced RIMM-18 cells change morphology, acquiring epithelial-like features, and begin to express epithelial markers (e.g., E-cadherin, cytokeratin, gamma-glutamyl-transpeptidase, and secreted frizzled-related **protein** 2 (sFRP2). Conclusion: This preliminary characterization of the RIMM-18 cell line suggests that it will be useful in the study of biochemical and molecular mechanisms of nephronic development and, possibly, of some types of renal cancer such as Wilms' tumor, which caricatures the normal process of kidney development.

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17226508 BIOSIS NO.: 200300185227

Optical bioluminescence and positron emission tomography imaging of a novel **fusion** reporter gene in tumor xenografts of living mice.

AUTHOR: Ray Pritha; Wu Anna M; Gambhir Sanjiv S (Reprint)

AUTHOR ADDRESS: David Geffen School of Medicine, Crump Institute for Molecular Imaging, University of California at Los Angeles, 700 Westwood Plaza, BRI B3-399A, P. O. Box 951770, Los Angeles, CA, 90095-1770, USA**
USA

AUTHOR E-MAIL ADDRESS: sgambhir@mednet.ucla.edu

JOURNAL: Cancer Research 63 (6): p1160-1165 March 15, 2003 2003

MEDIUM: print

ISSN: 0008-5472 (ISSN print)

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Noninvasive imaging of reporter gene expression using various imaging modalities is playing an increasingly important role in defining molecular events in the field of cancer biology, cell biology, and gene therapy. In this study, a novel reporter vector was constructed encoding a **fusion protein** comprised of a mutant herpes simplex virus type 1 thymidine kinase (HSV1-sr39tk) (tk), a positron emission tomography (PET) reporter gene, and renilla **luciferase** (rl), a bioluminescence optical reporter gene joined by a 20 amino acid long spacer sequence. We validated the activity of the two enzymes encoded by the **fusion protein** (tk20rl) in cell culture. Then, tumors stably expressing the tk20rl **fusion** gene were imaged both by microPET and optically using a cooled charge coupled device camera in xenograft-bearing living mice. Using a single **fusion** reporter (PET/optical) gene should accelerate the validation of reporter gene approaches developed in cell culture for translation into preclinical and clinical models.

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17041878 BIOSIS NO.: 200300000597

A Renilla **luciferase**-Aequorea GFP (ruc-gfp) **fusion** gene

construct permits real-time detection of promoter activation by exogenously administered mifepristone in vivo.

AUTHOR: Yu Y A; Szalay A A (Reprint)

AUTHOR ADDRESS: Department of Biochemistry, Loma Linda University School of Medicine, Loma Linda, CA, 92350, USA**USA

AUTHOR E-MAIL ADDRESS: aszalay@som.llu.edu

JOURNAL: MGG Molecular Genetics and Genomics 268 (2): p169-178 October 2002 2002

MEDIUM: print

ISSN: 1617-4615 (ISSN print)

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: In this study, we used a steroid-induced promoter activation system as a molecular switch to study the exogenous activation of transgene expression. This promoter activation system consists of three components: (1) a steroidal inducer drug, mifepristone (RU486), which binds to (2) a **chimeric** transcription factor complex, consisting of the mutant human progesterone receptor fused to the yeast GAL4 DNA-binding domain and the activation domain of the herpes simplex virus **protein** VP16, and (3) a synthetic promoter, consisting of a series of GAL4 recognition sequences upstream of the adenovirus major late ElB TATA box, linked to a gene construct (ruc-gfp) encoding a Renilla **luciferase**-Aequorea green fluorescent **protein** (GFP) **fusion** **protein**. Transcription of the promoter-marker gene cassette is activated by the drug (mifepristone)-bound **chimeric** transcription factor complex. Monitoring of induced gene expression was carried out using a low-light video camera and a UV microscope to detect **luciferase** and GFP, respectively. Using this activation system, we observed a 10- to 25-fold activation, depending on the inducer dose, of both **luciferase** and GFP expression in transiently transfected cells in comparison to cells that were not exposed to mifepristone. We further demonstrated activation of gene expression from the promoter activation system in live animals. The plasmids PAP CMV-GL914VPc'SV, carrying the **chimeric** transcription factor cassette, and plasmid p17X4-TATA-ruc-gfp, carrying the ruc-gfp reporter gene construct, were co-injected into limb muscles of nude mice. Following DNA injection, mifepristone (50 mug/kg) was delivered by intraperitoneal injection. Thirty-six hours after DNA and mifepristone injection, significant Renilla **luciferase** activity was detectable in the limb muscles. The promoter activation system was also demonstrated in limb muscles and livers of nude mice that had received transplants of ex vivo-modified cells, which were transiently transformed with both the **chimeric** activator plasmid and the ruc-gfp reporter plasmid prior to implantation. Significant Renilla activity and GFP fluorescence were detected externally in limb muscles and in the livers of anesthetized animals that had received an intraperitoneal injection of inducer. This external monitoring method for observing inducible gene expression in live animals will facilitate experimental studies of fundamental questions of biological and therapeutic relevance. It will be especially valuable for the analysis of gene function at specific stages of **animal** development. The method should also be of general use in gene therapy, since it permits simultaneous monitoring of the expression levels of light-emitting proteins and therapeutic proteins originating from the activation of identical promoters.

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17041874 BIOSIS NO.: 200300000593

Renilla **luciferase**-Aequorea GFP (Ruc-GFP) **fusion** **protein**,
a novel dual reporter for real-time imaging of gene expression in cell
cultures and in live animals.

AUTHOR: Wang Y; Yu Y A; Shabahang S; Wang G; Szalay A A (Reprint)

AUTHOR ADDRESS: Department of Biochemistry, Loma Linda University, Loma
Linda, CA, 92350, USA**USA

AUTHOR E-MAIL ADDRESS: aszalay@som.llu.edu

JOURNAL: MGG Molecular Genetics and Genomics 268 (2): p160-168 October
2002 2002

MEDIUM: print

ISSN: 1617-4615 (ISSN print)

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Light-emitting reporter proteins play an increasing role in the
study of gene expression in vitro and in vivo. Here we present a **ruc-gfp**
fusion gene construct generated by fusing a cDNA for Renilla
luciferase (**ruc**) in-frame with a cDNA encoding the 'humanized' GFP
(**gfp**) from Aequorea. A plasmid containing the **fusion** gene construct
was successfully transformed into, and expressed in, mammalian cells. The
transformed cells exhibited both Renilla **luciferase** activity in the
presence of coelenterazine and GFP fluorescence upon excitation with UV
light. Spectrofluorometry of cells containing the **Ruc-GFP fusion**
protein, in the absence of wavelengths capable of exciting GFP
fluorescence but in the presence of the **luciferase** substrate,
coelenterazine, showed an emission spectrum with two peaks at 475 nm and
508 nm. These two peaks correspond to the emission maximum of Renilla
luciferase at 475 nm and that of GFP at 508 nm. The peak at 508 nm
generated in the presence of coelenterazine alone (without UV excitation)
is the result of intramolecular energy transfer from Renilla
luciferase to Aequorea GFP. Southern analysis of genomic DNA
purified from transformed Chinese hamster ovary (CHO) cells and
fluorescence in situ hybridization (FISH) to metaphase chromosomes
confirmed the integration of the **ruc-gfp fusion** gene on a single
chromosome. The bifunctional **Ruc-GFP fusion protein** allows
the detection of gene expression at the single-cell level based on green
fluorescence, and in a group of cells based on luminescence emission.
Furthermore, **animal** experiments revealed that light emission from
the **Ruc-GFP fusion protein** can be detected externally in the
organs or tissues of live animals bearing the gene construct.

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16946764 BIOSIS NO.: 200200540275

The circadian clock that controls gene expression in Arabidopsis is tissue
specific

AUTHOR: Thain Simon C; Murtas Giovanni; Lynn James R; McGrath Robert B;

Millar Andrew J (Reprint)
AUTHOR ADDRESS: Department of Biological Sciences, University of Warwick,
Coventry, CV4 7AL, UK**UK
JOURNAL: Plant Physiology (Rockville) 130 (1): p102-110 September, 2002
2002
MEDIUM: print
ISSN: 0032-0889
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The expression of CHALCONE SYNTHASE (CHS) expression is an important control step in the biosynthesis of flavonoids, which are major photoprotectants in plants. CHS transcription is regulated by endogenous programs and in response to environmental signals. **Luciferase** reporter gene fusions showed that the CHS promoter is controlled by the circadian clock both in roots and in aerial organs of transgenic Arabidopsis plants. The period of rhythmic CHS expression differs from the previously described rhythm of chlorophyll a/b-binding **protein** (CAB) gene expression, indicating that CHS is controlled by a distinct circadian clock. The difference in period is maintained in the wild-type Arabidopsis accessions tested and in the de-etiolated 1 and timing of CAB expression 1 mutants. These clock-affecting mutations alter the rhythms of both CAB and CHS markers, indicating that a similar (if not identical) circadian clock mechanism controls these rhythms. The distinct tissue distribution of CAB and CHS expression suggests that the properties of the circadian clock differ among plant tissues. Several **animal** organs also exhibit heterogeneous circadian properties in culture but are believed to be synchronized in vivo. The fact that differing periods are manifest in intact plants supports our proposal that spatially separated copies of the plant circadian clock are at most weakly coupled, if not functionally independent. This autonomy has apparently permitted tissue-specific specialization of circadian timing.

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16895815 BIOSIS NO.: 200200489326
Ribozyme suppression of endogenous thyroid hormone receptor activity in
Xenopus laevis cells
AUTHOR: Lim Wayland; Furlow J David (Reprint)
AUTHOR ADDRESS: Section of Neurobiology, Physiology and Behavior, Division
of Biological Sciences, University of California, Davis, CA, 95616, USA**
USA
JOURNAL: Nucleic Acids Research 30 (15): p3490-3496 August 1, 2002 2002
MEDIUM: print
ISSN: 0305-1048
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Xenopus laevis is an excellent model for thyroid hormone (T3)-regulated gene expression. T3 initiates two drastically different pathways during metamorphosis: death of larval tissues and growth of adult tissues. The role that each T3 receptor (TR) isotype, alpha and

beta, plays in metamorphosis is uncertain. The *X. laevis* tetraploid genome limits experiments to overexpression, misexpression and dominant negative studies. Ribozymes offer an alternative by suppressing gene activity through specific mRNA reduction. It has been suggested that ribozymes will not work in *X. laevis* because of the organism's intracellular environment and body temperature. In this study, we show that hammer-head ribozymes are active in vitro against transcribed TRbeta message and in vivo against a TRbeta-~~luciferase fusion protein~~. We next show that TRbeta-targeted ribozymes can inhibit T3-induced transcription of a reporter gene in cultured *X. laevis* cells, using T3 response elements from two T3-responsive transcription factor genes. One has early expression kinetics in response to T3 and is proposed to be TRalpha regulated whereas the other has intermediate induction kinetics and thus may be partially TRbeta regulated. Therefore, ribozymes are a potentially valuable tool for overcoming the limitations in this system for examining gene function in *X. laevis*.

not organism

5/7/28

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16878139 BIOSIS NO.: 200200471650

Transcriptional repression of the rat osteocalcin gene by deltaEF1

AUTHOR: Sooy Karen; Demay Marie B (Reprint)

AUTHOR ADDRESS: Massachusetts General Hospital, 50 Blossom Street, Wellman
501, Boston, MA, 02114, USA**USA

JOURNAL: Endocrinology 143 (9): p3370-3375 September, 2002 2002

MEDIUM: print

ISSN: 0013-7227

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Intron I of the rat osteocalcin gene contains silencer elements that suppress osteocalcin-reporter ~~fusion~~ gene transcription. The consensus sequence for the transcription factor deltaEF1 is homologous to two pyrimidine-rich repeats in intron 1 that contribute to silencing of osteocalcin-reporter ~~fusion~~ genes. To assess if overexpression of deltaEF1 augments transcriptional repression by these sequences, the intron 1 sequences (wtS) were placed upstream to the native rat osteocalcin promoter fused to a ~~luciferase~~ reporter gene (-306-OCluc). Coexpression of the wtS-(-306-OCluc) ~~fusion~~ gene with deltaEF1 decreased ~~luciferase~~ activity 30% relative to cotransfection with empty vector. Repression was abolished by point mutations in the putative deltaEF1 motifs, mS-(-306-OCluc). To determine whether deltaEF1 binds to these DNA sequences, gel retardation assays were performed using oligonucleotides containing the putative osteocalcin deltaEF1 motifs and a classical deltaEF1 motif, as radiolabeled probes. A comigrating DNA-~~protein~~ complex generated by these probes was recognized by an antibody directed against deltaEF1 and competed for by excess unlabeled wild-type oligonucleotides. Oligonucleotides with mutations in the osteocalcin sequences, which abolish suppression, and in the deltaEF1 consensus site, that abolishes binding to deltaEF1, were unable to compete for the formation of this complex. Overexpression of deltaEF1 in ROS 17/2.8 cells led to an 84% decrease in osteocalcin mRNA levels relative to cells transfected with empty vector, confirming that

deltaEF1 suppresses expression of the endogenous osteocalcin gene.

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16848055 BIOSIS NO.: 200200441566

Gene therapy by intrahepatic and intratumoral trafficking of p53-VP22 induces regression of liver tumors

AUTHOR: Zender Lars; Koeck Reiner; Eckhard Matthias; Frericks Bernd; Goesling Thomas; Gebhardt Thomas; Drobek Susanne; Galanski Michael; Kuehnel Florian (Reprint); Manns Michael; Kubicka Stefan (Reprint)

AUTHOR ADDRESS: Department of Gastroenterology, Medical School Hannover, Carl Neuberg Strasse 1, 30625, Hannover, Germany**Germany

JOURNAL: Gastroenterology 123 (2): p608-618 August, 2002 2002

MEDIUM: print

ISSN: 0016-5085

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Background and Aims: VP22-mediated intercellular transport provides an approach to deliver functional **chimeric** proteins into a high percentage of target cells. The aim of this study was to evaluate the efficacy of p53/VP22 **fusion** **protein** in gene therapy of liver tumors. Methods: Expression vectors of N- and C-terminal **fusion** proteins of p53 and VP22 were subcloned and transcriptional properties of **chimeric** proteins were assessed by **luciferase** assays. Adenoviral vectors expressing p53 wild type (AdGFP/p53wt) and p53-VP22 (AdGFP/p53-VP22) were generated to investigate the VP22-mediated spreading in normal liver and in liver tumors in vivo by green fluorescent **protein** fluorescence and p53 immunohistochemistry. Gene therapy was investigated in subcutaneous and preclinical orthotopic **animal** tumor models after subcutaneous and intra-arterial administration of the adenoviruses, and tumor growth was assessed by direct calibration and magnetic resonance imaging. Results: p53-VP22 showed enhanced transcriptional activity compared with p53 wild type. VP22-mediated intercellular transport of p53 could be observed in the normal liver and in liver tumors in vivo and was correlated with increased antitumor efficacy of gene therapy and improved survival of the animals. Conclusions: **Fusion** of VP22 to p53 strongly improves the results of p53 replacement gene therapy. Furthermore, the demonstrated VP22-mediated intercellular transport in the liver could be important for other strategies in liver gene therapy, providing a tool for enhancing the effect of gene therapy in liver diseases such as metabolic disorders or viral hepatitis.

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16665873 BIOSIS NO.: 200200259384

Expression of fused luxAB gene of bacterial **luciferase** in liver carcinoma cells

AUTHOR: Mao Canquan (Reprint); Yang Shude (Reprint); Zhao Mei (Reprint); et

al

AUTHOR ADDRESS: Beijing Hospital, Beijing, 100730, China**China
JOURNAL: Zhonghua Zhongliu Zazhi 23 (5): p359-362 September, 2001 2001
MEDIUM: print
ISSN: 0253-3766
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: Chinese

ABSTRACT: Objective: This work was done to look into the expression of fused luxAB gene of bacterial **luciferase** as a reporter gene in liver carcinoma cells. Methods: The mammalian expression vector pcDNA3-luxAB gene, constructed by the **fusion** of luxA and luxB genes, were amplified in the polymerase chain reaction (PCR) directed site mutagenesis from the Vibrio harveyi 1600 strain and inserted into the plasmid of pcDNA3. This analysis was to confirm the fused luxAB gene and the positive clones obtained by the G418 resistant stable selection and transfected by lipofectin, when they were confirmed by the PCR. The growth curve of cell population and luminescence of bacterial **luciferase** was obtained through MTT and bioluminescence, respectively. Results: The fused luxAB gene, being a monocistron, completely agreed with the design. No significant difference in the growth curves of cell population was observed between the transfected cells and untransfected ones. The recombinant plasmid was likely to be expressed in a stable fashion in the BEL7402 cell. Meanwhile, the maximum cellular level in terms of vitro bioluminescent strength reached the point of (8.71+-1.21) mV/40 mug **protein**. Conclusion: Bacterial **luciferase** luxAB gene may become the first choice as a new, sensitive and non-invasive reporter gene in the research on liver cancer cells.

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16659855 BIOSIS NO.: 200200253366
YY1 activates Msx2 gene independent of bone morphogenetic **protein** signaling
AUTHOR: Tan D P; Nonaka K; Nuckolls G H; Liu Y H; Maxson R E; Slavkin H C; Shum L (Reprint)
AUTHOR ADDRESS: Craniofacial Development Section, National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health, 6 Center Drive, Building 6, Room 324, Bethesda, MD, 20892, USA**USA
JOURNAL: Nucleic Acids Research 30 (5): p1213-1223 March 1, 2002 2002
MEDIUM: print
ISSN: 0305-1048
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Msx2 is a homeobox gene expressed in multiple embryonic tissues which functions as a key mediator of numerous developmental processes. YY1 is a bi-functional zinc finger **protein** that serves as a repressor or activator to a variety of promoters. The role of YY1 during embryogenesis remains unknown. In this study, we report that Msx2 is

regulated by YY1 through **protein**-DNA interactions. During embryogenesis, the expression pattern of YY1 was observed to overlap in part with that of Msx2. Most notably, during first branchial arch and limb development, both YY1 and Msx2 were highly expressed, and their patterns were complementary. To test the hypothesis that YY1 regulates Msx2 gene expression, P19 embryonal cells were used in a number of expression and binding assays. We discovered that, in these cells, YY1 activated endogenous Msx2 gene expression as well as Msx2 promoter-**luciferase** **fusion** gene activity. These biological activities were dependent on both the DNA binding and activation domains of YY1. In addition, YY1 bound specifically to three YY1 binding sites on the proximal promoter of Msx2 that accounted for this transactivation. Mutations introduced to these sites reduced the level of YY1 transactivation. As bone morphogenetic **protein** type 4 (BMP4) regulates Msx2 expression in embryonic tissues and in P19 cells, we further tested whether YY1 is the mediator of this BMP4 activity. BMP4 did not induce the expression of YY1 in early mouse mandibular explants, nor in P19 cells, suggesting that YY1 is not a required mediator of the BMP4 pathway in these tissues at this developmental stage. Taken together, these findings suggest that YY1 functions as an activator for the Msx2 gene, and that this regulation, which is independent of the BMP4 pathway, may be required during early mouse craniofacial and limb morphogenesis.

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16616308 BIOSIS NO.: 200200209819

Monitoring the anti tumor activity of expanded CD8+ NKT cells after allogeneic bone marrow transplantation using bioluminescent imaging
AUTHOR: Edinger Matthias (Reprint); Verneris Michael R (Reprint); Cao Yuan; Bachmann Michael; Contag Christopher H; Negrin Robert S (Reprint)
AUTHOR ADDRESS: Division of Bone Marrow Transplantation, Stanford University School of Medicine, Stanford, CA, USA**USA
JOURNAL: Blood 98 (11 Part 1): p433a November 16, 2001 2001
MEDIUM: print
CONFERENCE/MEETING: 43rd Annual Meeting of the American Society of Hematology, Part 1 Orlando, Florida, USA December 07-11, 2001; 20011207
SPONSOR: American Society of Hematology
ISSN: 0006-4971
DOCUMENT TYPE: Meeting; Meeting Abstract; Meeting Poster
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Since light is transmitted through mammalian tissues at low levels, cells expressing the bioluminescent reporter gene **luciferase** (Luc) can be detected within living mice using low light imaging CCD cameras. Using this technique, we recently established **animal** models of leukemia and lymphoma, allowing us to localize and quantify tumor cells non-invasively in vivo. For that purpose, the tumor cell lines BCL1 and A20 were retrovirally transduced with a dual optical reporter gene containing. Luc together with green or yellow fluorescent **protein** (GFP or YFP) as a **fusion** construct. While the bioluminescent reporter gene Luc allowed the detection and quantification of the cells in vivo, the fluorescent reporter genes were used to

re-isolate tumor cells from the organs using flow cytometry. Luc expressing cells could be detected in internal organs such as spleen, liver, lung, lymph nodes and even within the bone marrow of living mice and the signal intensity detected in vivo correlated to tumor load. Measuring light emission over time in individual animals revealed the kinetics of tumor growth and regression after treatment. Due to the high sensitivity of the detection method it was possible to evaluate tumor cell trafficking and expansion even in minimal residual disease stages. Using this method the therapeutic application of a cytotoxic cell population, previously described as cytokine induced killer cells (CIK) was investigated. These cells were generated from splenocytes by stimulation with IFN-gamma on day 0, followed by anti-CD3-Ab and IL-2 stimulation and expansion over 2 wks. The resulting cell population is CD3+, CD8+ with 30-50% co-expressing the NK marker NK 1.1 (CD8+ NKT cells). The anti-tumor activity of these cells was examined in syngeneic tumor models as well as after allogeneic bone marrow transplantation (BMT). After allogeneic BMT (C57BL/6 into Balb/c) CIK cells inhibited the regrowth of the BCL1-luc lymphoma with a reduced propensity to induce GVHD as compared to allogeneic splenocytes. Animals treated with 2.5-10X10⁶ CIK cells showed long term survival of 85% and 30%, respectively, while, animals treated with as few as 1-2.5X10⁶ splenocytes all succumbed to acute GVHD by day 40 and 14, respectively. Animals transplanted with allogeneic BM only all succumbed to relapse from the BCL1 lymphoma, as shown by dramatic increases in bioluminescent signal intensity. Injection of transduced CIK cells expressing the **luciferase** gene into syngeneic animals bearing a subcutaneous lymphoma revealed that CIK cells trafficked to the tumor site within 3 days and remained there until the tumor was eradicated. We conclude, that this imaging technology allows for unique insights into tumor biology and immunology, since it is now possible to evaluate tumor responses and effector cell trafficking non-invasively even at minimal disease stages.

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16605539 BIOSIS NO.: 200200199050

Novel **fusion** of AML1 to Ubiquitin-Specific Protease 25 in myelodysplasia

AUTHOR: Ramsey Heather C (Reprint); Richkind Kathleen; Zaman Masood; Hromas Robert A

AUTHOR ADDRESS: Walther Oncology Center, Indiana University, Indianapolis, IN, USA**USA

JOURNAL: Blood 98 (11 Part 1): p561a November 16, 2001 2001

MEDIUM: print

CONFERENCE/MEETING: 43rd Annual Meeting of the American Society of Hematology, Part 1 Orlando, Florida, USA December 07-11, 2001; 20011207

SPONSOR: American Society of Hematology

ISSN: 0006-4971

DOCUMENT TYPE: Meeting; Meeting Abstract; Meeting Poster

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: AML1 (CBFalpha2) is a transcription factor that is essential for normal hematopoietic development. It interacts with CBFbeta to function as a heterodimeric transcriptional activator termed Core Binding Factor

(CBF), which is a master regulator of the genetic cascade responsible for myeloid differentiation. Gene activation targets of CBF include myeloperoxidase, neutrophil elastase, T-cell receptor genes, macrophage colony stimulating factor (M-CSF) receptor, and IL-3. AML1 is the most common target for translocations in acute myelogenous leukemia (AML) and has also been found to be a frequent target for translocation in myelodysplasia. Using rapid amplification of cDNA ends (RACE); we isolated a novel AML1 ~~fusion~~ transcript in two patients with myelodysplasia. The AML1 transcript isolated was a ~~fusion~~ between AML1 after exon 5 to the Ubiquitin Specific Protease 25 (USP25) located at 21q11. This transcript has AML1 fused out of frame to USP25 at nt 752, which is the 5' end of the USP25 exon 5. The USP25 gene provides only 6 additional amino acids before termination secondary to the out of frame ~~fusion~~. Thus, the remainder of USP25 was not translated in the same frame as AML1, resulting in a truncated AML1 ~~protein~~ that contained the DNA binding domain but not the transactivation domain. FISH analysis of both patients found that the AML1 locus was disrupted by translocation. Yet RACE PCR isolated intact AML1 transcripts in addition to the AML1-USP25 ~~fusion~~ transcript. Potential mechanisms for the generation of this transcript include an interstitial deletion/insertion, chromosomal cross-over, or alternative splicing. It is possible that this truncated ~~protein~~ could compete with normal CBFalpha2 and could function as a dominant negative inhibitor of hematopoietic promoters that CBF activates. In order to test this hypothesis, the AML1-USP25 ~~fusion~~ DNA was subcloned into the expression vector pcDNA3.1 and co-transfected with normal CBF and the M-CSF receptor promoter in a ~~Luciferase~~ reporter construct. The results showed that CBF activation of the M-CSF receptor promoter was inhibited by 2.7-fold by AML1-USP25. In addition, we tested whether AML1-USP25 could inhibit the synergistic activation of the M-CSF receptor promoter by CBF in combination with PU.1. AML1-USP25 also inhibited the synergistic activation of the M-CSF receptor promoter by CBF and PU.1 by 2.8-fold. This data implies that this AML1-USP25 ~~fusion~~ could inhibit hematopoietic differentiation, but another mutation may be needed for producing proliferation.

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16588693: BIOSIS NO.: 200200182204

Imaging of light emission from the expression of luciferases in living cells and organisms: A review

AUTHOR: Greer Lee F; Szalay Aladar A (Reprint)

AUTHOR ADDRESS: Department of Biochemistry, School of Medicine and
Department of Natural Sciences-Biology Section, Loma Linda University,
Loma Linda, CA, 92354, USA**USA

JOURNAL: Luminescence (Chichester) 17 (1): p43-74 January-February, 2002

MEDIUM: print

ISSN: 1522-7235

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Luciferases are enzymes that emit light in the presence of oxygen

and a substrate (luciferin) and which have been used for real-time, low-light imaging of gene expression in cell cultures, individual cells, whole organisms, and transgenic organisms. Such luciferin-luciferase systems include, among others, the bacterial lux genes of terrestrial *Photobacterium luminescens* and marine *Vibrio harveyi* bacteria, as well as eukaryotic luc and ruc genes from firefly species (*Photinus*) and the sea pansy (*Renilla reniformis*), respectively. In various vectors and in fusion constructs with other gene products such as green fluorescence protein (GFP; from the jellyfish *Aequorea*), luciferases have served as reporters in a number of promoter search and targeted gene expression experiments over the last two decades. Luciferase imaging has also been used to trace bacterial and viral infection in vivo and to visualize the proliferation of tumour cells in animal models.

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16557379 BIOSIS NO.: 200200150890

Nonsurgical direct delivery of plasmid DNA into rat heart: Time course, dose response, and the influence of different promoters on gene expression

AUTHOR: Sarkar Nondita; Blomberg Pontus (Reprint); Wardell Eva;

Eskandarpour Malihe; Sylven Christer; Drvota Viktor; Islam Khalid B

AUTHOR ADDRESS: Gene Therapy Center, The Clinical Research Center, Huddinge University Hospital, Stockholm, Sweden**Sweden

JOURNAL: Journal of Cardiovascular Pharmacology 39 (2): p215-224 February, 2002 2002

MEDIUM: print

ISSN: 0160-2446

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Transfer of genes encoding therapeutic proteins into the myocardium shows great potential for treatment of coronary artery disease. To quantitatively elucidate the behavior of plasmid DNA following cardiac gene transfer, time kinetics, dose-response relationship, systemic spread to the liver, and the influence of different promoters on plasmid DNA gene expression in rat hearts were examined using a novel nonsurgical direct delivery method that enables testing of large numbers of animals. Plasmids encoding either vascular endothelial growth factor A165 or a fusion protein between enhanced green fluorescent protein (EGFP) and luciferase were injected directly in rat hearts under echocardiographic guidance. The results show that gene expression is dose related and that the duration of gene expression is transient. These findings underscore the necessity to explore other efficient vectors or alternative methods of gene delivery to achieve increased and prolonged gene expression.

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16496681 BIOSIS NO.: 200200090192

Detection of GDNF secretion in glial cell culture and from transformed cell implants in the brains of live animals

AUTHOR: Liu H; Iacono R P; Szalay A A (Reprint)

AUTHOR ADDRESS: Department of Biochemistry, Loma Linda University School of Medicine, Loma Linda, CA, 92350, USA**USA

JOURNAL: MGG Molecular Genetics and Genomics 266 (4): p614-623 December, 2001 2001

MEDIUM: print

ISSN: 1617-4615

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Current ex vivo gene therapy for Parkinson's disease using glial cell line-derived neurotrophic factor (GDNF) is limited by the lack of a monitoring mechanism to determine the expression of GDNF once the cells or other vehicles are transferred into animal models. The purpose of this study was to test whether a Renilla luciferase (RUC)-GDNF fusion protein secreted by the genetically engineered glial cell line RG-1 could be measured photometrically in cerebrospinal fluid (CSF). RG-1 was constructed by permanent transformation with a plasmid DNA construct that contains a GDNF cDNA (gdnf) fused to a RUC cDNA (ruc). The fusion protein secreted by RG-1 was shown to retain both GDNF and RUC activity. The concentration of GDNF determined by enzyme-linked immunoadsorbent assay (ELISA) was correlated with the light emission detected by assaying for RUC bioluminescence in RG-1 culture medium, indicating that RUC can be used as a reporter for GDNF in vitro. The cells were then implanted into rat brain (n=20), and the cisternal CSF was analyzed. Bioluminescence was successfully detected in the CSF samples, and was quantified over a period of 25 days, while Western blotting and ELISA failed to detect GDNF in CSF, presumably because the concentration of the RUC-GDNF fusion was too low. This study demonstrates that the transformed glial cell line RG-1 offers a sensitive self-reporting assay for GDNF expression.

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16367473 BIOSIS NO.: 200100539312

Transcriptional activity of Hoxa 6 gene promoter

AUTHOR: Tan D P (Reprint); Shao X (Reprint); Peterkofsky A (Reprint)

AUTHOR ADDRESS: Lab. of Biochemical Genetics, NHLBI, NIH, Bethesda, MD, USA
**USA

JOURNAL: Society for Neuroscience Abstracts 27 (1): p1234 2001 2001

MEDIUM: print

CONFERENCE/MEETING: 31st Annual Meeting of the Society for Neuroscience
San Diego, California, USA November 10-15, 2001; 20011110

ISSN: 0190-5295

DOCUMENT TYPE: Meeting; Meeting Abstract

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Homeobox (Hox) genes play crucial roles during animal neurogenesis. Perturbation of expression of Hox genes can cause birth

defects, cancer, or other diseases. The mode of regulation of Hox genes at the promoter level is still unclear. We cloned and sequenced 6.3 kb of chromosomal DNA and a cDNA containing the complete mouse Hoxa 6 gene. This gene, containing 2 exons and 1 intron, encodes a **protein** of 232 amino acids. The expression of Hoxa 6 was detected in mRNA prepared from mouse embryos, embryonal P19 cells and NIH3T3 cells. For Hoxa 6 promoter analysis, a series of Hoxa 6 promoter-**Luciferase** **fusion** genes were created and transfected into P19 cells. **Luciferase** activity of Hoxa 6-Luc was measured and normalized to the activity of co-transfected pCMV-beta-galactosidase. 3.1 kb of the 5'-upstream region (-3.1 kb to +1 bp) of Hoxa 6 had the highest transcriptional activity. The 1.8, 1.24, 0.8 kb and 328 bp upstream regions also had significant activity. However, the fragment of -3.1 kb to -328 bp failed to show any transcriptional activity. These data suggested that an essential activity of the Hoxa 6 promoter resided in the proximal 328 bp fragment. Deletion of 168 bp within this fragment caused an 8-fold reduction of transcription activity suggesting the existence of enhancer elements in this fragment. Point mutation of the SP1 binding site within this 168 bp fragment reduced activity 2-fold. Further deletion of a GGGC repeat caused no further reduction. In summary, the main promoter region of Hoxa 6 is located in a 328 bp proximal fragment and the SP1 site is required for full expression of Hoxa 6. Other elements, which may act together with the SP1 site, are required for optimum activity of the Hoxa 6 promoter.

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16230330 BIOSIS NO.: 200100402169

Identification of activating transcription factor 4 (ATF4) as an Nrf2-interacting **protein**: Implication for heme oxygenase-1 gene regulation

AUTHOR: He Chuan Hua; Gong Pengfei; Hu Bin; Stewart Daniel; Choi Mary E; Choi Augustine M K; Alam Jawed (Reprint)

AUTHOR ADDRESS: Dept. of Molecular Genetics, Alton Ochsner Medical Foundation, 1516 Jefferson Hwy., New Orleans, LA, 70121, USA**USA

JOURNAL: Journal of Biological Chemistry 276 (24): p20858-20865 June 15, 2001 2001

MEDIUM: print

ISSN: 0021-9258

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Nrf2 regulates expression of genes encoding enzymes with antioxidant (e.g. heme oxygenase-1 (HO-1)) or xenobiotic detoxification (e.g. NAD(P)H:quinone oxidoreductase, glutathione S-transferase) functions via the stress- or antioxidant-response elements (StRE/ARE). Nrf2 heterodimerizes with small Maf proteins, but the role of such dimers in gene induction is controversial, and other partners may exist. By using the yeast two-hybrid assay, we identified activating transcription factor (ATF) 4 as a potential Nrf2-interacting **protein**. Association between Nrf2 and ATF4 in mammalian cells was confirmed by co-immunoprecipitation and mammalian two-hybrid assays. Furthermore, Nrf2cntdotATF4 dimers bound to an StRE sequence from the ho-1 gene.

CdCl2, a potent inducer of HO-1, increased expression of ATF4 in mouse hepatoma cells, and detectable induction of ATF4 protein preceded that of HO-1 (30 min versus 2 h). A dominant-negative mutant of ATF4 inhibited basal and CdCl2-stimulated expression of a StRE-dependent/luciferase fusion construct (pE1-luc) in hepatoma cells but only basal expression in mammary epithelial MCF-7 cells. A dominant mutant of Nrf2 was equally inhibitory in both cell types in the presence or absence of CdCl2. These results indicate that ATF4 regulates basal and CdCl2-induced expression of the ho-1 gene in a cell-specific manner and possibly in a complex with Nrf2.

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16162380 BIOSIS NO.: 200100334219

Recombinant expression of biologically active rat leptin in Escherichia coli

AUTHOR: Park Jung-Hyun; Lee Hyun-Hee; Na Shin-Young; Ju Sung-Kyu; Lee Yun-Jung; Lee Myung-Kyu; Kim Kil Lyong (Reprint)

AUTHOR ADDRESS: Protein Engineering Laboratory, Korea Research Institute of Bioscience and Biotechnology, Yusong, Taejon, 305-600, South Korea**South Korea

JOURNAL: Protein Expression and Purification 22 (1): p60-69 June, 2001

MEDIUM: print

ISSN: 1046-5928

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Leptin is a 16-kDa nonglycosylated hormone that is produced in mature adipocytes and which acts primarily in the hypothalamus to reduce food intake and body weight. While the rat is a representative laboratory animal model in obesity research, so far recombinant rat leptin was not available. In the present study, rat leptin was recombinantly expressed in Escherichia coli and purified in a bioactive form to provide a further tool for the analysis of leptin functions in rats. Leptin cDNA was cloned by RT-PCR from total RNA of SD rat adipocytes, and overexpression was achieved by subcloning the leptin cDNA into the pET-29a vector, which enabled the recombinant expression of rat leptin as an S-peptide-tagged fusion protein. Since the fusion proteins were expressed in inclusion bodies, after purification of the insoluble fraction, leptin proteins were refolded by sequential dialysis into physiological buffers. The biological activity of this recombinant protein was confirmed in proliferation assays using leptin-sensitive rat insulinoma cells as well as a newly developed leptin-sensitive luciferase assay system. The specific binding of the S-tagged leptin to leptin-receptor-expressing cells was further shown by flow cytometry using fluorescence-conjugated S-proteins.

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16128367 BIOSIS NO.: 200100300206

Visualizing leukemia and lymphoma cell homing and quantification of tumor burden in response to therapy in living animals

AUTHOR: Edinger Matthias (Reprint); Verneris Michael R (Reprint); Cao Yuan (Reprint); Bachmann Michael H (Reprint); Costa Gina L (Reprint); Contag Christopher H (Reprint); Negrin Robert S (Reprint)

AUTHOR ADDRESS: Departments of Medicine and Pediatrics, Stanford University, School of Medicine, Stanford, CA, USA**USA

JOURNAL: Blood 96 (11 Part 1): p123a November 16, 2000 2000

MEDIUM: print

CONFERENCE/MEETING: 42nd Annual Meeting of the American Society of Hematology San Francisco, California, USA December 01-05, 2000; 20001201

SPONSOR: American Society of Hematology

ISSN: 0006-4971

DOCUMENT TYPE: Meeting; Meeting Abstract; Meeting Poster

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Revealing the mechanisms of neoplastic disease and enhancing our ability to intervene in these processes requires an increased understanding of cellular and molecular changes as they occur in living animals. We have previously shown, that since light is transmitted through mammalian tissues at a low level, we can detect light emission from tumor cells that express **luciferase** from within living animals, using low light imaging cameras. To establish **animal** models of leukemia and lymphoma, we used the A20 and Bcl1 lymphoma cell lines. A20 cells were transfected with the pCDNA3.1-luc plasmid containing a modified **luciferase** gene (A20-luc). Using light emission as an indicator, tumor engraftment, exponential growth and rejection was followed in individual animals after sc, iv and ip injections. Tumor engraftment in spleen, liver and lymph nodes could be localized. As few as 103 A20-luc cells could be detected after sc injection. Following iv injection of 104 A20-luc cells in a syngeneic BMT model, leukemic disease was observed with tumor infiltration of the femurs, humeri, sternum, vertebrae and skull. A retroviral transduction system was used to transfer the gene for a GFP/luc **fusion protein** to the Bcl1 lymphoma, known to localize to liver and spleen. GFP expressing cells were sorted by FACS and injected into mice iv. Initially, as few as 7000 tumor cells could be detected, and subsequently tumor engraftment in liver and spleen was observed. Tumor growth was followed over time and quantified from these internal organs. We also investigated the therapeutic application of cytotoxic cells termed cytokine induced killer cells (CIK) which co-express T cell and NK cell markers and are generated by in vitro expansion from splenocytes. Treatment of A20-luc tumor bearing animals with 107 CIK cells led to a decrease of tumor signal from sc growing tumors and 75% of the mice were cured within 2 wks. To examine trafficking of these activated T cells we used the retroviral transduction method to label the cells. After iv injection transduced cells were detected in the lungs, 12 h later they locate to liver and spleen, and generalize thereafter. We conclude, that whole body imaging of labeled tumor cells reveals patterns of systemic disease allowing visualization of minimal disease and therapeutic response. This will improve our insights in tumor biology, be useful in directing subsequent ex vivo assays, and provide a powerful tool to examine the kinetics of response to novel therapies.

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16115320 BIOSIS NO.: 200100287159
HSV infection upregulates Fas Ligand expression
AUTHOR: Leibole M A (Reprint); Herndon J M (Reprint); Ferguson T A
(Reprint)
AUTHOR ADDRESS: Ophthalmology Vis Sci, Washington Univ, Saint Louis, MO,
USA**USA
JOURNAL: IOVS 42 (4): pS42 March 15, 2001 2001
MEDIUM: print
CONFERENCE/MEETING: Annual Meeting of the Association for Research in
Vision and Ophthalmology Fort Lauderdale, Florida, USA April 29-May 04,
2001; 20010429
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Citation
LANGUAGE: English

5/7/42
DIALOG(R)File 5:Biosis Previews(R)
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16085753 BIOSIS NO.: 200100257592
Translational silencing of monocytic cell expression of the iron transport
%%protein%% ceruloplasmin (Cp)
AUTHOR: Mazumder Barsan (Reprint); Seshadri Vasudevan (Reprint); Fox Paul L
(Reprint)
AUTHOR ADDRESS: Cleveland Clinic, 9500 Euclid Ave., Cleveland, OH, 44195,
USA**USA
JOURNAL: FASEB Journal 15 (5): pA975 March 8, 2001 2001
MEDIUM: print
CONFERENCE/MEETING: Annual Meeting of the Federation of American Societies
for Experimental Biology on Experimental Biology 2001 Orlando, Florida,
USA March 31-April 04, 2001; 20010331
ISSN: 0892-6638
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: A role of the copper %%protein%% Cp in iron metabolism is suggested by its ferroxidase activity, by anemia in copper-deficient patients and animals, and by iron overload in patients with hereditary Cp deficiency. The liver is the major source of Cp, but the monocyte/macrophage is a secondary source. Interferon (IFN)-gamma induces ceruloplasmin (Cp) synthesis by U937 monocytic cells; however, synthesis stops abruptly after about 16 h by a mechanism involving transcript-specific translational silencing. We have shown previously that binding of a cytosolic factor(s) to a cis-element in the Cp mRNA 3'-UTR is required for translational silencing. We here investigate, by translation of %%chimeric%% reporter transcripts in rabbit reticulocyte lysates, the role of the poly(A) tail in this translational control mechanism. Translation of a %%luciferase%% (luc) transcript upstream of the Cp 3'-UTR and a poly (A) tail (luc-Cp 3'-UTR-poly(A)) was blocked by cytosol from IFN-treated cells. However, the cytosol did not inhibit translation of a transcript lacking poly (A). A similar requirement for

poly(A) was shown for endogenous Cp mRNA; translational silencing was not observed after the poly(A) tail was removed by RNaseH treatment. The mechanism by which binding of a factor(s) to the 3'-UTR influences translation initiation at the distant 5'-UTR is not known. The addition of antibodies against poly(A)-binding **protein** (PABP) or initiation factor eIF4G almost completely rescued the in vitro translation of Luc-Cp-3'-UTR-poly(A) in the presence of the inhibitory cell extract, thus showing the requirement for interactions between the 5'- and 3'-termini. One possible explanation is suggested by the recently described "circular" or "closed-loop" model of mRNA translation. According to this model, efficient translation depends on functional interactions between PABP and the poly(A) tail, and with factor eIF4G of the initiation complex. On the basis of this model, and our new findings, we suggest that circularization of the Cp mRNA is required for optimal interaction of a silencing factor with the initiation complex. This silencing mechanism may minimize adverse consequences of Cp-mediated alterations to iron homeostasis during inflammation.

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16082670 BIOSIS NO.: 200100254509

Developmental regulation of mitogen-activated **protein**

kinase-activated kinases-2 and -3 (MAPKAPK-2/-3) in vivo during corpus luteum formation in the rat

AUTHOR: Maizels Evelyn T; Mukherjee Abir; Sithanandam Gunamani; Peters Carl A; Cottom Joshua; Mayo Kelly E; Hunzicker-Dunn Mary (Reprint)

AUTHOR ADDRESS: Department of Cell and Molecular Biology, Northwestern University Medical School, 303 East Chicago Avenue, Chicago, IL, 60611-3008, USA**USA

JOURNAL: Molecular Endocrinology 15 (5): p716-733 May, 2001 2001

MEDIUM: print

ISSN: 0888-8809

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: The current study investigates the activation in vivo and regulation of the expression of components of the p38 mitogen-activated **protein** kinase (MAPK) pathway during gonadotropin-induced formation and development of the rat corpus luteum, employing a sequential PMSG/human CG (hCG) treatment paradigm. We postulated that the p38 MAPK pathway could serve to promote phosphorylation of key substrates during luteal maturation, since maturing luteal cells, thought to be cAMP-nonresponsive, nevertheless maintain critical phosphoproteins. Both p38 MAPK and its upstream activator MAPK kinase-6 (MKK6) were found to be chronically activated during the luteal maturation phase, with activation detected by 24 h post hCG and maintained through 4 days post hCG. The p38 MAPK downstream **protein** kinase target termed MAPK-activated **protein** kinase-3 (MAPKAPK-3) was newly induced at both mRNA and **protein** levels during luteal formation and maturation, while mRNA and **protein** expression of the closely related MAPKAPK-2 diminished. Two potential substrates for MAPKAPKs, the small heat shock **protein** HSP-27 and the cAMP regulatory element binding **protein** CREB, were monitored in vivo for phosphorylation. HSP-27 phosphorylation was not

modulated during luteal maturation. In contrast, we observed sustained luteal-phase CREB phosphorylation in vivo, consistent with upstream MKK6/p38 MAPK activation and MAPKAPK-3 induction. MAPKAPK-3-specific immune complex kinase assays provided direct evidence that MAPKAPK-3 was in an activated state during luteal maturation in vivo. Cellular inhibitor studies indicated that an intact p38 MAPK path was required for CREB phosphorylation in a cellular model of luteinization, as treatment of luteinized granulosa cells with the p38 MAPK inhibitor SB 203580 strongly inhibited CREB phosphorylation. Transient transfection studies provided direct evidence that MAPKAPK-3 was capable of signaling to activate CREB transcriptional activity, as assessed by means of GAL4-CREB fusion protein construct coexpressed with GAL4-luciferase reporter construct. Introduction of wild-type, but not kinase-dead mutant, MAPKAPK-3 cDNA, into a mouse ovarian cell line stimulated GAL4-CREB-dependent transcriptional activity approximately 3-fold. Thus MAPKAPK-3 is indeed uniquely poised to support luteal maturation through the phosphorylation and activation of the nuclear transcription factor CREB.

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15925313 BIOSIS NO.: 200100097152

Cis- and trans-acting factors controlling acetylcholinesterase mRNA stability during myogenic differentiation

AUTHOR: Belanger G (Reprint); Chan R Y; Jasmin B J

AUTHOR ADDRESS: University of Ottawa, Ottawa, ON, Canada**Canada

JOURNAL: Society for Neuroscience Abstracts 26 (1-2): pAbstract No.-411.7
2000 2000

MEDIUM: print

CONFERENCE/MEETING: 30th Annual Meeting of the Society of Neuroscience New Orleans, LA, USA November 04-09, 2000; 20001104

SPONSOR: Society for Neuroscience

ISSN: 0190-5295

DOCUMENT TYPE: Meeting; Meeting Abstract

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Expression of several mRNAs, including those encoding synaptic proteins, are known to increase markedly during myogenic differentiation. For example, levels of acetylcholinesterase (AChE) mRNA increase by at least 10-fold upon fusion of myoblasts into myotubes. These changes in AChE expression appear regulated at the transcriptional (see accompanying abstract by Angus, Chan and Jasmin) as well as at the post-transcriptional levels (Fuentes and Taylor, Neuron 10: 679-687, 1993). In the present study, we examined several mechanisms that may be involved in regulating the stability of AChE transcripts in differentiating C2 cells by focusing on the 3'UTR of the shorter AChE mRNA expressed in skeletal muscle cells. Initially, we determined the polyadenylation state of AChE transcripts using the RACE-PAT assay. No difference in the length of the poly(A)+ tail was observed in myoblasts versus myotubes. Transfection of C2 cells with a luciferase expression vector containing the AChE 3'UTR in either the sense or antisense orientation demonstrated that this region is important for controlling the stability of AChE transcripts in myogenic cells since the

5/7/45

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15849201 BIOSIS NO.: 200100021040

In vitro and in vivo functional analysis of human T cell lymphotropic virus type 1 pX open reading frames I and II

AUTHOR: Lairmore Michael D (Reprint); Albrecht Bjorn; D'Souza Celine; Nisbet John W; Ding Wei; Bartoe Joshua T; Green Patrick L; Zhang Weiqing

AUTHOR ADDRESS: Department of Veterinary Biosciences, Ohio State University, 1925 Coffey Road, Columbus, OH, 43210-1093, USA**USA

JOURNAL: AIDS Research and Human Retroviruses 16 (16): p1757-1764 November 1, 2000 2000

MEDIUM: print

ISSN: 0889-2229

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

chimeric mRNA appeared destabilized in myoblasts. We also examined whether cytoplasmic factors interact with the AChE 3'UTR. To this end, we generated 3 fragments of various lengths from the 3'UTR. UV-crosslinking experiments using 100 mug of proteins revealed different patterns of RNA-***protein*** interactions in myoblasts versus myotubes. In addition, these experiments indicated that specific regions in the 3'UTR, that are conserved across species, are critically involved in binding ***protein*** complexes. The pattern of interactions also suggests that the binding of cytoplasmic factors may be both sequence- and structure-specific. These results illustrate the importance of cis- and trans-acting factors acting on the 3'UTR of AChE transcripts to regulate their abundance in differentiating myogenic cells.

ABSTRACT: Human T lymphotropic virus type 1 (HTLV-1) is a complex retrovirus containing regulatory and accessory genes encoded in four open reading frames (ORF I-IV) of the pX region. It is not clear what role pX ORFs I and II-encoded proteins have in the pathogenesis of the lymphoproliferative diseases associated with HTLV-1 infection. The conserved ORF I encodes for a hydrophobic 12-kDa ***protein***, p12, I that contains four SH3 binding motifs (PXXP) that localizes to cellular endomembranes when overexpressed in cultured cells. Differential splicing of pX ORF II results in the production of two nuclear proteins, p13II and p30II. p13II also localizes to mitochondria. p30II shares homology with the POU family of transcription factors. We have identified functional roles of pX ORF I and ORF II in establishment and maintenance of infection in a rabbit model. To functionally study p12I we have tested a proviral clone with selective ablation of ORF I (ACH.p12I) for its ability to infect quiescent peripheral blood mononuclear cells (PBMC). Our data indicate that T cells infected with the wild-type clone of HTLV-1 (ACH) are more efficient than ACH.p12I in infecting quiescent PBMC. These findings parallel our ***animal*** model data and suggest a role for p12I in the activation of quiescent lymphocytes, a prerequisite for effective viral replication in vivo. To test the ability of p30II to function as a transcription factor we have constructed p30II as a Gal4-***fusion*** ***protein***. When transfected with Gal4-driven ***luciferase*** reporter genes, the p30II-Gal4-***fusion*** ***protein*** induces transcriptional activity up to 50-fold in both 293 and HeLa-Tat cells.

These systems will be useful to identify molecular mechanisms that explain the functional role of pX ORF I and ORF II-encoded proteins in HTLV-1 replication.

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15702294 BIOSIS NO.: 200000420607

Xlim-1 and LIM domain binding protein 1 cooperate with various transcription factors in the regulation of the goosecoid promoter
AUTHOR: Mochizuki Toshiaki; Karavanov Alexander A; Curtiss Patricia E; Ault Katherine T; Sugimoto Naoshi; Watabe Tetsuro; Shiokawa Koichiro; Jamrich Milan; Cho Ken W Y; Dawid Igor B; Taira Masanori (Reprint)

AUTHOR ADDRESS: Laboratory of Molecular Embryology, Department of Biological Sciences, Graduate School of Science, University of Tokyo, Bunkyo-ku, Tokyo, 113-0033, Japan**Japan

JOURNAL: Developmental Biology 224 (2): p470-485 August 15, 2000 2000

MEDIUM: print

ISSN: 0012-1606

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: The homeobox genes Xlim-1 and goosecoid (gsc) are coexpressed in the Spemann organizer and later in the prechordal plate that acts as head organizer. Based on our previous finding that gsc is a possible target gene for Xlim-1, we studied the regulation of gsc transcription by Xlim-1 and other regulatory genes expressed at gastrula stages, by using gsc-luciferase reporter constructs injected into animal explants. A 492-bp upstream region of the gsc promoter responds to Xlim-1/3m, an activated form of Xlim-1, and to a combination of wild-type Xlim-1 and Ldb1, a LIM domain binding protein, supporting the view that gsc is a direct target of Xlim-1. Footprint and electrophoretic mobility shift assays with GST-homeodomain fusion proteins and embryo extracts overexpressing FLAG-tagged full-length proteins showed that the Xlim-1 homeodomain or Xlim-1/Ldb1 complex recognize several TAATXY core elements in the 492-bp upstream region, where XY is TA, TG, CA, or GG. Some of these elements are also bound by the ventral factor PV.1, whereas a TAATCT element did not bind Xlim-1 or PV.1 but did bind the anterior factors Otx2 and Gsc. These proteins modulate the activity of the gsc reporter in animal caps: Otx2 activates the reporter synergistically with Xlim-1 plus Ldb1, whereas Gsc and PV.1 strongly repress reporter activity. We show further, using animal cap assays, that the endogenous gsc gene was synergistically activated by Xlim-1, Ldb1, and Otx2 and that the endogenous otx2 gene was activated by Xlim-1/3m, and this activation was suppressed by the posterior factor Xbra. Based on these data, we propose a model for gene interactions in the specification of dorsoventral and anteroposterior differences in the mesoderm during gastrulation.

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15601203 BIOSIS NO.: 200000319516

Similar poly(C)-sensitive RNA-binding complexes regulate the stability of the heavy and light neurofilament mRNAs

AUTHOR: Canete-Soler Rafaela (Reprint); Schlaepfer William W

AUTHOR ADDRESS: Division of Neuropathology, University of Pennsylvania, Philadelphia, PA, 19104, USA**USA

JOURNAL: Brain Research 867 (1-2): p265-279 9 June, 2000 2000

MEDIUM: print

ISSN: 0006-8993

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: The potential role of RNA processing in regulating neurofilament (NF) subunit expression and in mediating the neuropathic effects of NF transgenes was explored by determining whether similar regulatory elements and cognate binding factors are present in NF mRNAs. Gel-shift studies were used to compare RNA-binding complexes that assemble on the 3'UTR of the heavy (NF-H), mid-sized (NF-M) and light (NF-L) NF mRNAs when radioactive RNA probes are incubated with high-speed supernatants (S100) of rat brain homogenates. RNA-binding complexes were characterized by their rate of migration in non-denaturing gels and by their ability to be competed with specific homoribopolymers. Similar RNA-binding complexes formed on probes to the 3'UTRs of NF-L and NF-H mRNAs. The complexes were competed with poly(C) and are referred to as poly(C)-sensitive complexes. Their binding sites were localized to a 36 nt sequence in the mid-distal region of the NF-H 3'UTR and to a 45 nt sequence at the proximal edge of the 3'UTR of the NF-L transcript. Although the binding sites showed limited sequence homology, the complexes were cross-competed with unlabeled probes and radioactivity in either probe was cross-linked to a 43 kDa ***protein***. The 43 kDa ***protein*** also bound directly to NF-L and NF-H probes in Northwestern blots. Functional studies showed that deletion of the binding sites markedly increased expression of a ***luciferase*** reporter gene containing the 3'UTR of NF-L or NF-H by stabilizing the ***fusion*** transcripts. Point mutations in the NF-H binding site which prevented formation of the poly(C)-sensitive complex also stabilized the ***fusion*** mRNA. The findings reveal a common destabilizing element in the 3'UTR of NF-L and NF-H mRNAs that may be important in coordinating NF subunit expression and in mediating the neuropathic effects of the NF-L and NF-H transgenes in transgenic mice.

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15315952 BIOSIS NO.: 200000034265

Survival, physiology, and lysis of Lactococcus lactis in the digestive tract

AUTHOR: Drouault Sophie; Corthier Gerard; Dusko Ehrlich S; Renault Pierre (Reprint)

AUTHOR ADDRESS: Unite de Genetique Microbienne, Institut National de la Recherche Agronomique, 78352, Jouy en Josas cedex, France**France

JOURNAL: Applied and Environmental Microbiology 65 (11): p4881-4886 Nov., 1999 1999

MEDIUM: print

ISSN: 0099-2240

DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The survival and the physiology of lactococcal cells in the different compartments of the digestive tracts of rats were studied in order to know better the fate of ingested lactic acid bacteria after oral administration. For this purpose, we used strains marked with reporter genes, the luxA-luxB gene of *Vibrio harveyi* and the gfp gene of *Aequora victoria*, that allowed us to differentiate the inoculated bacteria from food and the other intestinal bacteria. %%Luciferase%% was chosen to measure the metabolic activity of *Lactococcus lactis* in the digestive tract because it requires NADH, which is available only in metabolically active cells. The green fluorescent %%protein%% was used to assess the bacterial lysis independently of death. We report not only that specific factors affect the cell viability and integrity in some digestive tract compartments but also that the way bacteria are administrated has a dramatic impact. Lactococci which transit with the diet are quite resistant to gastric acidity (90 to 98% survival). In contrast, only 10 to 30% of bacteria survive in the duodenum. Viable cells are metabolically active in each compartment of the digestive tract, whereas most dead cells appear to be subject to rapid lysis. This property suggests that lactococci could be used as a vector to deliver specifically into the duodenum the proteins produced in the cytoplasm. This type of delivery vector would be particularly appropriate for targeting digestive enzymes such as lipase to treat pancreatic deficiencies.

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14829277 BIOSIS NO.: 199900088937

CCR5-mediated human immunodeficiency virus entry depends on an amino-terminal gpl20-binding site and on the conformational integrity of all four extracellular domains

AUTHOR: Genoud Stephane; Kajumo Francis; Guo Yong; Thompson Daniah; Dragic Tatjana (Reprint)

AUTHOR ADDRESS: Aaron Diamond AIDS Res. Cent., 455 1st Ave., 7th Floor, New York, NY 10016, USA**USA

JOURNAL: Journal of Virology 73 (2): p1645-1648 Feb., 1999 1999

MEDIUM: print

ISSN: 0022-538X

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: The human immunodeficiency virus type 1 coreceptor activity of CCR5 depends on certain polar and charged residues in its amino-terminal domain. Since studies of %%chimeric%% receptors have indicated that the extracellular loops of CCR5 are also involved in viral %%fusion%% and entry, we have explored the role of bulky, polar and nonpolar residues in these regions. Selected amino acids in the three extracellular loops were individually changed to alanines, and the coreceptor activities of the mutant CCR5 proteins were tested in a %%luciferase%% reporter virus-based entry assay. We found that the cysteines in the extracellular loops of CCR5 are essential for coreceptor activity. However, only minor

(two- to threefold) effects on coreceptor function were noted for all of the other alanine substitutions. We also demonstrated that when the first 19 residues of the amino-terminal region were separated from the rest of CCR5, by insertion of glycine/serine spacers between proline 19 and cysteine 20, coreceptor function decreased. Together with our previous studies, these data indicate that both an amino-terminal gp120-binding site and extracellular domain geometry play a role in viral entry.

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14118757 BIOSIS NO.: 199799752817

High expression of naked plasmid DNA in muscles of young rodents

AUTHOR: Danko I; Williams P; Herweijer H; Zhang G; Latendresse J S; Bock I; Wolff J A (Reprint)

AUTHOR ADDRESS: Univ. Wisconsin-Madison, Dep. Pediatrics, Waisman Cent., Madison, WI 53705, USA**USA

JOURNAL: Human Molecular Genetics 6 (9): p1435-1443 1997 1997

ISSN: 0964-6906

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: There is a time window at 2 weeks of age for achieving very high levels of foreign gene expression from the intramuscular injection of naked plasmid DNA in mice and rats. The highest expression, over 1 μ -g of **luciferase** **protein**/muscle, was obtained in Balb/C mice using constructs containing the CMV promoter, a **chimeric** intron and the luc+ **luciferase** gene. Approximately 50% of the myofibers were intensely blue following the intramuscular injection of a beta-galactosidase expression vector in 2 week old Balb/C mice. The effects of age, mouse strain and construct were multiplicative, resulting in gt 1000-fold greater **luciferase** and -20-fold more beta-galactosidase-positive cells. These high levels of expression were unstable and were not observed in larger animals (dog, rhesus monkey). These results indicate that enormous levels of foreign gene expression can be obtained in muscle with naked DNA in vivo and will enable the temporary effects of gene function and expression in rodent muscle to be expeditiously studied.

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12348952 BIOSIS NO.: 199497370237

Chimeric receptors as a tool for luminescent measurement of biological activities of steroid hormones

AUTHOR: Jausons-Loffreda N (Reprint); Balaguer P; Roux S; Fuentes M; Pons M; Nicolas J-C; Gelmini S; Pazzagli M

AUTHOR ADDRESS: INSERM Unite 58, 60 rue de Navacelles, 34090 Montpellier, France**France

JOURNAL: Journal of Bioluminescence and Chemiluminescence 9 (3): p217-221 1994 1994

ISSN: 0884-3996

DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Some applications of %%chimeric%% cellular models are presented to study the biological activities of steroid hormones. We have used several %%chimeric%% constructs encoding the DNA binding domain of Gal4 yeast %%protein%% fused to the hormone binding domain of various steroid receptors (MR, PR, GR and ER). Interactions of these %%chimeric%% receptors with a 17-mer DNA sequence, specific for Gal-4, control expression of the firefly %%luciferase%% as a reporter gene. Stable transfected cell lines expressing the firefly %%luciferase%% under the control of different steroids were established and an efficient and easy sub-cloning was allowed with the help of an imaging system using a single-photoncounting camera. In the cell lines obtained, the bioluminescent response can be easily measured and thus used to measure specific biological activities of steroid agonists or antagonists. We observed that the responses are effector-concentration-dependent and their biological activities will be compared to those of native receptors.

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11233338 BIOSIS NO.: 199293076229
FIREFLY %%LUCIFERASE%% AS A MARKER FOR HERPESVIRUS PSEUDORABIES VIRUS
REPLICATION IN-VITRO AND IN-VIVO
AUTHOR: KOVACS F S (Reprint); METTENLEITER T C
AUTHOR ADDRESS: VET MED RES INST, HUNGARIAN ACAD SCI, PO BOX 18, H-1581
BUDAPEST, HUNG**HUNGARY
JOURNAL: Journal of General Virology 72 (12): p2999-3008.1991
ISSN: 0022-1317
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Insertion of reporter genes into complex viral genomes and monitoring virus replication by detecting the corresponding %%protein%% products is increasingly used in pathogenesis studies. We present here the isolation and characterization of a recombinant neurotrophic alpha-herpesvirus, pseudorabies virus (PrV), which stably carries the gene encoding firely %%luciferase%%. To express the enzyme the complete open reading frame for %%luciferase%% was fused to the promoter and first seven codons of the non-essential glycoprotein gX gene of PrV. A recombinant PrV carrying the %%luciferase%% gene inserted into the gX gene and exhibiting strong %%luciferase%% activity after infection of cultured cells was further characterized. Kinetic analyses showed that %%luciferase%% activity was detectable as early as 90 min after infection. %%Luciferase%% expression could be monitored in cell extracts in a luminometer. For facilitating plaque isolation of %%luciferase%% recombinant viruses it was also visualized in situ on sensitive film. Kinetic experiments in mice proved the suitability of %%luciferase%% as an excellent marker for following herpesvirus spread in the %%animal%%. By way of %%luciferase%% detection we show that PrV invasion of the central nervous system after intranasal infection of

mice occurred independently of replication in non-neural tissues such as lung or thymus. Furthermore, comparison of iosgenic %%%luciferase%% recombinaant PrV strains carrying intact or deleted glycoprotein gI genes showed differences in the organotropism between these two viruses.

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09645267 BIOSIS NO.: 198987093158

HIGHLY ATTENUATED VACCINIA VIRUS MUTANTS FOR THE GENERATION OF SAFE RECOMBINANT VIRUSES

AUTHOR: RODRIGUEZ D (Reprint); RODRIGUEZ J-R; RODRIGUEZ J F; TRAUBER D; ESTEBAN M

AUTHOR ADDRESS: DEP BIOCHEM, STATE UNIV NY HEALTH SCI CENT, BROOKLYN, NY 11203, USA**USA

JOURNAL: Proceedings of the National Academy of Sciences of the United States of America 86 (4): p1287-1291 1989

ISSN: 0027-8424

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: ENGLISH

ABSTRACT: An anttenuated vaccinia virus mutant with specific genetic lesions has been used to develop a vehicle for safer live recombinant virus vaccines. The mutant virus 48-7 has an 8-MDa deletion starting 2.2 MDa from the left end of the viral genome and point mutations in the gene encoding the 14-kDa %%%fusion%% %%%protein%% that determines the plaque-size phenotype of the virus. Using the highly sensitive reporter gene %%%luciferase%%, we have shown that this mutant can generate recombinant viruses that infect cultured cells and animals with normal vaccinia virus tropism. Insertion of the envelope and gag genes of human immunodeficiency virus type 1 into the attenuated vaccinia mutant resulted in their efficient expression and precursor processing in infected cultured cells. Infection of mice with human immunodeficiency virus-vaccinia recombinant viruses elicited human immunodeficiency virus-specific antibodies. Using mice pretreated with cyclophosphamide as a model for immunosuppression, the reduced virulence of the mutant recombinant virus was clearly evident. These findings demonstrate that the highly attenuated vaccinia virus mutant 48-7 can be used to generate effective and safer vaccines.

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